COMPOSITIONS, REAGENTS AND KITS FOR AND METHODS OF DIAGNOSING AND TREATING OBESITY AND/OR DIABETES

FIELD OF THE INVENTION

The present invention relates to obesity and diabetes markers, to reagents which can detect obesity and diabetes marker transcripts and translation products, to kits and methods for detecting obesity and diabetes marker transcripts and translation products, to methods and kits for screening and diagnosing obesity and diabetes in individuals and monitoring response to treatment, disease progression and disease recurrence in patients diagnosed with obesity and diabetes, to compounds which specifically bind to translation products of obesity and/or diabetes marker transcripts, to compositions for and methods of treating obesity and/or diabetes.

BACKGROUND OF THE INVENTION

Obesity is the second most important cause of preventable death in the United States, exceeded only by cigarette smoking. Obesity is estimated to affect 58,000,000 people and contribute to 300,000 deaths annually in the United States and its prevalence is increasing. Individuals suffering from the disease are at increased risk of illness from hypertension, lipid disorders, coronary heart disease, type II diabetes, stroke, gall bladder disease, osteoarthritis, sleep apnea, respiratory problems and certain cancers.

Obesity develops when there is an excess of energy intake over energy usage. The causes of this excess may vary from patient to patient and are believed to stem from various genetic, social and environmental factors. Current research supports the view that under identical environmental conditions, different people gain weight

at different rates and the amount they gain seems to be genetically determined. It has been proposed that natural selection caused our distant ancestors to acquire 'thrifty genes' which boosted the ability to store fat from each feast in order to sustain the body through the next famine. In today's environment of a surfeit of high fat, high calorie 'western style' food, 'thrifty genes' have become a liability.

More and more scientists and physicians are coming to reject the traditional belief that poor diet and lack of exercise are solely to blame for obesity and are increasingly tending to view it as a medical condition. Health economists, using prospective studies and national health statistics, have calculated the costs of obesity in the US in 1995 at $99.2 billion. By 2005 it is estimated that more than 120 million will be obese. The number of people living in France, Germany, Italy, the UK and the US could rise from 71 million in 1999 to 78 million in 2005. The economic impact of obesity in the US is now comparable to that of diabetes and ranks alongside expenditure on heart disease and hypertension. Medical researchers calculate that at least 88% of all cases of type II diabetes, 57% of coronary heart disease cases, 11% of breast cancers, and 10% of colon cancers diagnosed in overweight Americans are attributable to obesity.

The World health Organization has classified the obesity condition as an epidemic, and has set up a special task force to tackle one of the greatest risks to human health and well-being.

There remains a need for obesity and/or diabetes specific markers. There remains a need for reagents and kits which can be used to detect the presence of obesity and/or diabetes markers in samples from patients. There remains a need for methods of screening and diagnosing individuals who have obesity and/or diabetes and methods of monitoring response to treatment, disease progression and disease recurrence in patients diagnosed with obesity and diabetes. There remains a need for reagents, kits and methods for determining the type of obesity and/or diabetes that an individual who is obese has. There remains a need for compositions which can specifically target obesity and/or diabetes related cells. There remains a need for imaging agents

which can specifically bind to obesity and/or diabetes cells. There remains a need for improved methods of imaging obesity and/or diabetes cells. There remains a need for therapeutic agents which can specifically bind to obesity and/or diabetes cells. There remains a need for improved methods of treating individuals who are suspected of suffering from obesity and diabetes.

GLOSSARY

In the following description and claims use will be made, at times, with a variety of terms, and the meaning of such terms as they should be construed in accordance with the invention is as follows:

"Obesity and/or diabetes nucleic acid sequences" – the sequence shown in any one of SEQ ID NO: 1 to SEQ ID NO: 4 and of SEQ ID NO: 22 to SEQ ID NO: 25 sequences having at least *90% identity* (see below) to said sequences and *fragments* (see below) of the above sequences of least 20 b.p. long. These sequences are sequences coding for naturally occurring, alternative splice variants of the native and known Adiponectin, depicted in Locus Link as locus Hs. 9370 under Accession Number NM_004797 which is the sequence coding for the human 30kDa glycoprotein of 244 amino acids. It should be emphasized that the novel variants of the present invention are naturally occurring sequences resulting from alternative splicing of Adiponectin and not merely truncated, mutated or fragmented form of the gene.

the sequence shown in any one of SEQ ID NO: 5 to SEQ ID NO: 9 and of SEQ ID NO: 26 to SEQ ID NO: 30 sequences having at least *90% identity* (see below) to said sequences and *fragments* (see below) of the above sequences of least 20 b.p. long. These sequences are sequences coding for naturally occurring, alternative splice variants of the native and known Adiponectin, depicted in Locus Link as locus Mm. 11450 under Accession Number NM_009605 which is the sequence coding for the mouse 30kDa glycoprotein of 247 amino acids. It should be emphasized that the novel

variants of the present invention are naturally occurring sequences resulting from alternative splicing of Adiponectin and not merely truncated, mutated or fragmented form of the gene.

the sequence shown in any one of SEQ ID NO: 10 to SEQ ID NO: 11 and of SEQ ID NO: 31 to SEQ ID NO: 32 sequences having at least *90% identity* (see below) to said sequences and *fragments* (see below) of the above sequences of least 20 b.p. long. These sequences are sequences coding for naturally occurring, alternative splice variants of the native and known Ghrelin, depicted in Locus Link as locus Hs. 51738 under Accession Number NM_016362 which is the sequence coding for the human 13kDa glycoprotein of 117 amino acids. It should be emphasized that the novel variants of the present invention are naturally occurring sequences resulting from alternative splicing of Ghrelin and not merely truncated, mutated or fragmented form of the gene.

the sequence shown in any one of SEQ ID NO: 12 to SEQ ID NO: 18 and of SEQ ID NO: 33 to SEQ ID NO: 39 sequences having at least *90% identity* (see below) to said sequences and *fragments* (see below) of the above sequences of least 20 b.p. long. These sequences are sequences coding for naturally occurring, alternative splice variants of the native and known 11-beta-HSD, depicted in Locus Link as locus Hs. 3290 under Accession Number NM_005525 which is the sequence coding for the human 32kDa glycoprotein of 292 amino acids. It should be emphasized that the novel variants of the present invention are naturally occurring sequences resulting from alternative splicing of 11-beta-HSD and not merely truncated, mutated or fragmented form of the gene.

the sequence shown in any one of SEQ ID NO: 19 to SEQ ID NO: 21 and of SEQ ID NO: 40 to SEQ ID NO: 42 sequences having at least *90% identity* (see below) to said sequences and *fragments* (see below) of the above sequences of least 20 b.p. long. These sequences are sequences coding for naturally occurring, alternative splice variants of the native and known 11-beta-HSD, depicted in Locus Link as locus Mm. 15483 under Accession Number NM_008288 which is the sequence coding for the

mouse 32kDa glycoprotein of 292 amino acids. It should be emphasized that the novel variants of the present invention are naturally occurring sequences resulting from alternative splicing of 11-beta-HSD and not merely truncated, mutated or fragmented form of the gene.

The description of the obesity and/or diabetes variants and their difference from the original sequence is summarized in Table 1 as follows:

Table 1

SEQ ID NO:	Obesity and Diabetes related genes	GenBank Human Locus ID	GenBank Mouse Locus ID	Gene Symbol	Variation description
1	Adiponectin-WT (Variant 1)	9370	11450	APM	Nucleotide sequence of the human wild type protein (human)
2	Adiponectin Variant 2				Nucleotide sequence of variant 2 (human)
3	Adiponectin Variant 3				Nucleotide sequence of variant 3 (human)
4	Adiponectin Variant 4				Nucleotide sequence of variant 4 (human)
5	Adiponectin-WT (Variant 1)	9370	11450	APM	Nucleotide sequence of the mouse wild type protein (mouse)
6	Adiponectin Variant 2				Nucleotide sequence of variant 2 (mouse)
7	Adiponectin Variant 3				Nucleotide sequence of variant 3 (mouse)
8	Adiponectin Variant 4				Nucleotide sequence of variant 4 (mouse)
9	Adiponectin Variant 5				Nucleotide sequence of variant 5 (mouse)
10	Ghrelin- WT (variant 1)	51738	58991	GHRL	Nucleotide sequence of the human wild type protein
11	Ghrelin Variant 2				Nucleotide sequence of variant 2 (human)
12	11-beta-HSD – WT (Variant 1)	3290	15483	HSD11B1	Nucleotide sequence of the wild type human protein
13	11-beta-HSD Variant 2				Nucleotide sequence of variant 2 (human)
14	11-beta-HSD Variant 3				Nucleotide sequence of variant 3 (human)
15	11-beta-HSD Variant 4				Nucleotide sequence of variant 4 (human)
16	11-beta-HSD Variant 5				Nucleotide sequence of variant 5 (human)
17	11-beta-HSD Variant 6				Nucleotide sequence of variant 6 (human)
18	11-beta-HSD Variant 7				Nucleotide sequence of variant 7 (human)
19	11-beta-HSD				Nucleotide sequence of the

	– WT (Variant 1)				mouse wild type protein
20	11-beta-HSD Variant 8				Nucleotide sequence of variant 8 (mouse)
21	11-beta-HSD Variant 9				Nucleotide sequence of variant 9 (mouse)
22	Adiponectin-WT (Variant 1)	9370	11450	APM	Wild type human protein sequence
23	Adiponectin Variant 2				Alternative initiation (human)
24	Adiponectin Variant 3				
25	Adiponectin Variant 4				
26	Adiponectin-WT (Variant 1)	9370	11450	APM	Wild type mouse protein sequence
27	Adiponectin Variant 2				Alternative initiation (mouse)
28	Adiponectin Variant 3				Alternative 45 amino acids from position 111 in the wild type protein creating a variant with 156 amino acids (mouse)
29	Adiponectin Variant 4				Alternative 58 amino acids from position 111 in the wild type protein creating a variant with 169 amino acids (mouse)
30	Adiponectin Variant 5				Truncated variant 76 amino acids long (mouse)
31	Ghrelin- WT (variant 1)	51738	58991	GHRL	Wild type human protein sequence
32	Ghrelin Variant 2				Alternative 70 amino acids from position 35 in the wild type protein creating a variant with 117 amino acids (human)
33	11-beta-HSD – WT (Variant 1)	3290	15483	HSD11B1	Wild type human protein sequence
34	11-beta-HSD Variant 2				Deletion of 18 amino acids from amino acid 64 in the wild type protein and an

					alternative exon of 16 amino acids replacing the rest of the amino acids from amino acid 165 in the wild type protein (human)
35	11-beta-HSD Variant 3				Alternative 9 amino acids from amino acid 286 creating a variant with 295 amino acids (human)
36	11-beta-HSD Variant 4				Deletion of 18 amino acids from amino acid 137 till amino acid 155 in the wild type protein (human)
37	11-beta-HSD Variant 5				Deletion of 20 amino acids from amino acid 64 till amino acid 84 in the wild type protein (human)
38	11-beta-HSD Variant 6				Alternative initiation at amino acid no. 31 in the wild type protein (human)
39	11-beta-HSD Variant 7				Deletion of 48 amino acids from amino acid 173 till amino acid 221 in the wild type protein (human)
40	11-beta-HSD – WT (Variant 1)				Wild type mouse protein sequence
41	11-beta-HSD Variant 8				Deletion of 32 amino acids from amino acid 29 till amino acid 71 in the wild type protein
42	11-beta-HSD Variant 9				Alternative 19 amino acids from amino acid 173 creating a variant with 192 amino acids (mouse)

SEQ ID NOS: 1-21 are nucleotide sequences.

SEQ ID NOS: 22-42 are protein sequences encoded by SEQ ID NOS 1-21.

Table 2

SEQ ID 1-9 Adiponectin variants:

SEQ ID NO. 1: NM_004797_T1 | Length 4517

CTGATTCCATACCAGAGGGGCTCAGGATGCTGTTGCTGGGAGCTGTTCTACTGCTATTAG

CTCTGCCCGGGCATGACCAGGAAACCACGACTCAAGGGCCCGGAGTCCTGCTTCCCCTGC

CCAAGGGGGCCTGCACAGGTTGGATGGCGGGCATCCCAGGGCATCCGGGCCATAATGGGG
CCCCAGGCCGTGATGGCAGAGATGGCACCCCTGGTGAGAAGGGTGAGAAAGGAGATCCAG
GTCTTATTGGTCCTAAGGGAGACATCGGTGAAACCGGAGTACCCGGGGCTGAAGGTCCCC
GAGGCTTTCCGGGAATCCAAGGCAGGAAAGGAGAACCTGGAGAAGGTGCCTATGTATACC
GCTCAGCATTCAGTGTGGGATTGGAGACTTACGTTACTATCCCCAACATGCCCATTCGCT
TTACCAAGATCTTCTACAATCAGCAAAACCACTATGATGGCTCCACTGGTAAATTCCACT
GCAACATTCCTGGGCTGTACTACTTTGCCTACCACATCACAGTCTATATGAAGGATGTGA
AGGTCAGCCTCTTCAAGAAGGACAAGGCTATGCTCTTCACCTATGATCAGTACCAGGAAA
ATAATGTGGACCAGGCCTCCGGCTCTGTGCTCCTGCATCTGGAGGTGGGCGACCAAGTCT
GGCTCCAGGTGTATGGGGAAGGAGAGCGTAATGGACTCTATGCTGATAATGACAATGACT
CCACCTTCACAGGCTTTCTTCTCTACCATGACACCAACTGATCACCACTAACTCAGAGCC
TCCTCCAGGCCAAACAGCCCCAAAGTCAATTAAAGGCTTTCAGTACGGTTAGGAAGTTGA
TTATTATTTAGTTGGAGGCCTTTAGATATTATTCATTCATTTACTCATTCATTTATTCAT
TCATTCATCAAGTAACTTTAAAAAAATCATATGCTATGTTCCCAGTCCTGGGGAGCTTCA
CAAACATGACCAGATAACTGACTAGAAAGAAGTAGTTGACAGTGCTATTTTGTGCCCACT
GTCTCTCCTGATGCTCATATCAATCCTATAAGGCACAGGGAACAAGCATTCTCCTGTTTT
TACAGATTGTATCCTGAGGCTGAGAGAGTTAAGTGAATGTCTAAGGTCACACAGTATTAA
GTGACAGTGCTAGAAATCAAACCCAGAGCTGTGGACTTTGTTCACTAGACTGTGCCCTTT
TATAGAGGTACATGTTCTCTTTGGAGTGTTGGTAGGTGTCTGTTTCCCACCTCACCTGAG
AGCCATTGAATTTGCCTTCCTCATGAATTAAAACCTCCCCCAAGCAGAGCTTCCTCAGAG
AAAGTGGTTCTATGATGAAGTCCTGTCTTGGAAGGACTACTACTCAATGGCCCCTGCACT
ACTCTACTTCCTCTTACCTATGTCCCTTCTCATGCCTTTCCCTCCAACGGGGAAAGCCAA
CTCCATCTCTAAGTGCTGAACTCATCCCTGTTCCTCAAGGCCACCTGGCCAGGAGCTTCT
CTGATGTGATATCCACTTTTTTTTTTTTTGAGATGGAGTCTCACTCTGTCACCCAGGCT
GGAGTACAGTGACACGACCTCGGCTCACTGCAGCCTCCTTCTCCTGGGTCCAAGCAATTA
TTGTGCCTCAGCCTCCCGAGTAGCTGAGACTTCAGGTGCATTCCACCACACATGGCTAAT
TTTTGTATTTTTAGTAGAAATGGGGTTTCGTCATGTTGGCCAGGCTGGTCTCGAACTCCT
GGCCTAGGTGATCCACCCGCCTCGACCTCCCAAAGTGCTGGGATTACAGGCATGAGCCAC
CATGCCCAGTCGATATCTCACTTTTTATTTTGCCATGGATGAGAGTCCTGGGTGTGAGGA
ACACCTCCCACCAGGCTAGAGGCAACTGCCCAGGAAGGACTGTGCTTCCGTCACCTCTAA
ATCCCTTGCAGATCCTTGATAAATGCCTCATGAAGACCAATCTCTTGAATCCCATATCTA
CCCAGAATTAACTCCATTCCAGTCTCTGCATGTAATCAGTTTTATCCACAGAAACATTTT
CATTTTAGGAAATCCCTGGTTTAAGTATCAATCCTTGTTCAGCTGGACAATATGAATCTT
TTCCACTGAAGTTAGGGATGACTGTGATTTTCAGAACACGTCCAGAATTTTTCATCAAGA
AGGTAGCTTGAGCCTGAAATGCAAAACCCATGGAGGAATTCTGAAGCCATTGTCTCCTTG
AGTACCAACAGGGTCAGGGAAGACTGGGCCTCCTGAATTTATTATTGTTCTTTAAGAATT
ACAGGTTGAGGTAGTTGATGGTGGTAAACATTCTCTCAGGAGACAATAACTCCAGTGATG
TTTTTCAAAGATTTTAGCAAAAACAGAGTAAATAGCATTCTCTATCAATATATAAATTTA
AAAAACTATCTTTTTGCTTACAGTTTTAAATTCTGAACAATTTCTCTTATATGTGTATTG
CTAATCATTAAGGTATTATTTTTTCCACATATAAAGCTTTGTCTTTTTGTTGTTGTTGTT
GTTTTTAAGATGGAGTTTCCCTCTGTTGCCAGGCTAGAGTGCAGTGGCATGATCTCGGCT
TACTGCAACCTTTGCCTCCCAGGTTTAAGCGATTCTTCTGCCTCAGCCTCCCGAGTAGCT
GGGACCACAGGTGCCTACCACCATGCCAGGCTAATTTTTGTATTTTTAGTAAAGACAGGG

TTTCACCATATTGGCCAGGCTGGTCTCGAACTCCTGACCTTGTGATCTGCCCGCCTCCAT
TGTGTTGTTATTTGTGAGAAAGATAGATATGAGGTTTAGAGAGGGATGAAGAGGTGAGAG
TAAGCCTTGTGTTAGTCAGAACTCTGTGTTGTGAATGTCATTCACAACAGAAAACCCAAA
ATATTATGCAAACTACTGTAAGCAAGAAAAATAAAGGAAAAATGGAAACATTTATTCCTT
TGCATAATAGAAATTACCAGAGTTGTTCTGTCTTTAGATAAGGTTTGAACCAAAGCTCAA
AACAATCAAGACCCTTTTCTGTATGTCCTTCTGTTCTGCCTTCCGCAGTGTAGGCTTTAC
CCTCAGGTGCTACACAGTATAGTTCTAGGGTTTCCCTCCCGATATCAAAAAGACTGTGGC
CTGCCCAGCTCTCGTATCCCCAAGCCACACCATCTGGCTAAATGGACATCATGTTTTCTG
GTGATGCCCAAAGAGGAGAGAGGAAGCTCTCTTTCCCAGATGCCCCAGCAAGTGTAACCT
TGCATCTCATTGCTCTGGCTGAGTTGTGTGCCTGTTTCTGACCAATCACTGAGTCAGGAG
GATGAAATATTCATATTGACTTAATTGCAGCTTAAGTTAGGGGTATGTAGAGGTATTTTC
CCTAAAGCAAAATTGGGACACTGTTATCAGAAATAGGAGAGTGGATGATAGATGCAAAAT
AATACCTGTCCACAACAAACTCTTAATGCTGTGTTTGAGCTTTCATGAGTTTCCCAGAGA
GACATAGCTGGAAAATTCCTATTGATTTTCTCTAAAATTTCAACAAGTAGCTAAAGTCTG
GCTATGCTCACAGTCTCACATCTGGTGGGGGTGGGCTCCTTACAGAACACGCTTTCACAG
TTACCCTAAACTCTCTGGGGCAGGGTTATTCCTTTGTGGAACCAGAGGCACAGAGACAGT
CAACTGAGGCCCAACAGAGGCCTGAGAGAAACTGAGGTCAAGATTTCAGGATTAATGGTC
CTGTGATGCTTTGAAGTACAATTGTGGATTTGTCCAATTCTCTTTAGTTCTGTCAGCTTT
TGCTTCATATATTTTAGCGCTCTATTATTAGATATATACATGTTTAGTATTATGTCTTAT
TGGTGCATTTACTCTCTTATCATTATGTAATGTCCTTCTTTATCTGTGATAATTTTCTGT
GTTCTGAAGTCTACTTTGTCTAAAAATAACATACGCACTCAACTTCCTTTTCTTTCTTCC
TTCCTTTCTTTCTTCCTTCCTTTCTTTCTCTCTCTCTCTTTCCTTCCTTCCTTCCTCCTT
TTCTCTCTCTCTCTCTCTCTCTCTTTTCTTGACAGACTCTCGTTCTGTGGCCCTGGCT
GGAGTTCAGTGGTGTGATCTTGGCTCACTGCTACCTCTACCATGAGCAATTCTCCTGCCT
CAGCCTCCCAAGTAGCTGGAACTACAGGCTCATGCCACTGCGCCCAGCTAATTTTTGTAT
TTTTCGTAGAGACGGGGTTTCACCACATTCGTCAGGTTGGTTTCAAACTCCTGACTTTGT
GATCCACCCGCCTCGGCCTCCCAAAGTGCTGGGATTACAGGCATGAGCCATCACACCTGG
TCAACTTTCTTTTGATTAGTGTTTTTGTGGTATATCTTTTTCCATCATGTTACTTTAAAT
ATATCTATATTATTGTATTTAAAATGTGTTTCTTACAGACTGCATGTAGTTGGGTATAAT
TTTTATCCAGTCTAAAAATATCTGTCTTTTAATTGGTGTTTAGACAATTTATATTTAATA
AAATGGTGGAATTTAAA

SEQ ID NO. 2: NM_004797_T2 | Length 484

ATGACCCGGGGCTGAAGGTCCCCGAGGCTTTCCGGGAATCCAAGGCAGGAAAGGAGAACC
TGGAGAAGGTGCCTATGTATACCGCTCAGCATTCAGTGTGGGATTGGAGACTTACGTTAC
TATCCCCAACATGCCCATTCGCTTTACCAAGATCTTCTACAATCAGCAAAACCACTATGA
TGGCTCCACTGGTAAATTCCACTGCAACATTCCTGGGCTGTACTACTTTGCCTACCACAT
CACAGTCTATATGAAGGATGTGAAGGTCAGCCTCTTCAAGAAGGACAAGGCTATGCTCTT
CACCTATGATCAGTACCAGGAAAATAATGTGGACCAGGCCTCCGGCTCTGTGCTCCTGCA
TCTGGAGGTGGGCGACCAAGTCTGGCTCCAGGTGTATGGGGAAGGAGAGCGTAATGGACT
CTATGCTGATAATGACAATGACTCCACCTTCACAGGCTTTCTTCTCTACCATGACACCAA
CTGA

SEQ ID NO. 3: NM_004797_T3 | Length 718

CTGATTCCATACCAGAGGGGCTCAGGATGCTGTTGCTGGGAGCTGTTCTACTGCTATTAG

CTCTGCCCGGGCATGACCAGGAAACCACGACTCAAGGGCCCGGAGTCCTGCTTCCCCTGC
CCAAGGGGGCCTGCACAGGTTGGATGGCGGGCATCCCAGGGCATCCGGGCCATAATGGGG
CCCCAGGCCGTGATGGCAGAGATGGCACCCCTGGTGAGAAGGGTGAGAAAGGAGATCCAG
GTCTTATTGGTCCTAAGGGAGACATCGGTGAAACCGGAGTACCCGGGGCTGAAGGTCCCC
GAGGCTTTCCGGGAATCCAAGGCAGGAAAGGAGAACCTGGAGAAGGTGCGTTACTATCCC
CAACATGCCCATTCGCTTTACCAAGATCTTCTACAATCAGCAAAACCACTATGATGGCTC
CACTGGTAAATTCCACTGCAACATTCCTGGGCTGTACTACTTTGCCTACCACATCACAGT
CTATATGAAGGATGTGAAGGTCAGCCTCTTCAAGAAGGACAAGGCTATGCTCTTCACCTA
TGATCAGTACCAGGAAAATAATGTGGACCAGGCCTCCGGCTCTGTGCTCCTGCATCTGGA
GGTGGGCGACCAAGTCTGGCTCCAGGTGTATGGGGAAGGAGAGCGTAATGGACTCTATGC
TGATAATGACAATGACTCCACCTTCACAGGCTTTCTTCTCTACCATGACACCAACTGA

SEQ ID NO. 4: NM_004797_T4 | Length 537

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CCAAGGGGGCCTGCACAGGTTGGATGGCGGGCATCCCAGGGCATCCGGGCCATAATGGGG
CCCCAGGCCGTGATGGCAGAGATGGCACCCCTGGTGAGAAGGGTGAGAAAGGAGATCCAG
GTCTTATTGGTCCTAAGGGAGACATCGGTGAAACCGGAGTACCCGGGGCTGAAGGTCCCC
GAGGCTTTCCGGGAATCCAAGGCAGGAAAGGAGAACCTGGAGAAGGTGCCTATGTATACC
GCTCAGCATTCAGTGTGGGATTGGAGACTTACGTTACTATCCCCAACATGCCCATTCGCT
TTACCAAGATCTTCTACAATCAGCAAAACCACTATGATGGCTCCACTGGTAAATTCCACT
GCAACATTCCTGGGCTGTACCTTCACAGGCTTTCTTCTCTACCATGACACCAACTGA

SEQ ID NO. 5: U37222_T1 | Length: 1306 WT

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ACCAAAAGGGCTCAGGATGCTACTGTTGCAAGCTCTCCTGTTCCTCTTAATCCTGCCCAG
TCATGCCGAAGATGACGTTACTACAACTGAAGAGCTAGCTCCTGCTTTGGTCCCTCCACC
CAAGGGAACTTGTGCAGGTTGGATGGCAGGCATCCCAGGACATCCTGGCCACAATGGCAC
ACCAGGCCGTGATGGCAGAGATGGCACTCCTGGAGAGAAGGGAGAGAAAGGAGATGCAGG
TCTTCTTGGTCCTAAGGGTGAGACAGGAGATGTTGGAATGACAGGAGCTGAAGGGCCACG
GGGCTTCCCCGGAACCCCTGGCAGGAAAGGAGAGCCTGGAGAAGCCGCTTATGTGTATCG
CTCAGCGTTCAGTGTGGGGCTGGAGACCCGCGTCACTGTTCCCAATGTACCCATTCGCTT
TACTAAGATCTTCTACAACCAACAGAATCATTATGACGGCAGCACTGGCAAGTTCTACTG
CAACATTCCGGGACTCTACTACTTCTCTTACCACATCACGGTGTACATGAAAGATGTGAA
GGTGAGCCTCTTCAAGAAGGACAAGGCCGTTCTCTTCACCTACGACCAGTATCAGGAAAA
GAATGTGGACCAGGCCTCTGGCTCTGTGCTCCTCCATCTGGAGGTGGGAGACCAAGTCTG
GCTCCAGGTGTATGGGGATGGGGACCACAATGGACTCTATGCAGATAACGTCAACGACTC
TACATTTACTGGCTTTCTTCTCTACCATGATACCAACTGACTGCAACTACCCATAGCCCA
TACACCAGGAGAATCATGGAACAGTCGACACACTTTCAGCTTAGTTTGAGAGATTGATTT
TATTGCTTAGTTTGAGAGTCCTGAGTATTATCCACACGTGTACTCACTTGTTCATTAAAC
GACTTTATAAAAAATAATTTGTGTTCCTAGTCCAGAAAAAAAGGCACTCCCTGGTCTCCA
CGACTCTTACATGGTAGCAATAACAGAATGAAAATCACATTTGGTATGGGGGCTTCACAA
TATTCGCATGACTGTCTGGAAGTAGACCATGCTATTTTTCTGCTCACTGTACACAAATAT
TGTTCACATAAACCCTATAATGTAAATATGAAATACAGTGATTACTCTTCTCACAGGCTG
ASTGTATGAATTCTAAAGACCCATAAGTATTAAAGTGGTAGGGATAAATTGGAAAAAAAA

AAAAAAAAAAAGAAAAACTTTAGAGCACACTGGCGGCCGTTACTAG

SEQ ID NO. 6: U37222_T2 | LENGTH: 1184

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ATAGGAGCCTGCCAGGCCGTGGAGAGTGAGGAAGCAGAGATGACGGAGATGATGTCTTTC
CTTGTCCTGTGAAATGGATTGTGGGTAGAGGTTCCGGAGATAATGCCTCTTGCTGGAAAC
AGTCTGGGCAGTTCTGTTCCCGCCATTCACAGAATTCTTCTCACTTTCTAGGTCTTCTTG
GTCCTAAGGGTGAGACAGGAGATGTTGGAATGACAGGAGCTGAAGGGCCACGGGGCTTCC
CCGGAACCCCTGGCAGGAAAGGAGAGCCTGGAGAAGCCGCTTATGTGTATCGCTCAGCGT
TCAGTGTGGGGCTGGAGACCCGCGTCACTGTTCCCAATGTACCCATTCGCTTTACTAAGA
TCTTCTACAACCAACAGAATCATTATGACGGCAGCACTGGCAAGTTCTACTGCAACATTC
CGGGACTCTACTACTTCTCTTACCACATCACGGTGTACATGAAAGATGTGAAGGTGAGCC
TCTTCAAGAAGGACAAGGCCGTTCTCTTCACCTACGACCAGTATCAGGAAAAGAATGTGG
ACCAGGCCTCTGGCTCTGTGCTCCTCCATCTGGAGGTGGGAGACCAAGTCTGGCTCCAGG
TGTATGGGGATGGGGACCACAATGGACTCTATGCAGATAACGTCAACGACTCTACATTTA
CTGGCTTTCTTCTCTACCATGATACCAACTGACTGCAACTACCCATAGCCCATACACCAG
GAGAATCATGGAACAGTCGACACACTTTCAGCTTAGTTTGAGAGATTGATTTTATTGCTT
AGTTTGAGAGTCCTGAGTATTATCCACACGTGTACTCACTTGTTCATTAAACGACTTTAT
AAAAAATAATTTGTGTTCCTAGTCCAGAAAAAAAGGCACTCCCTGGTCTCCACGACTCTT
ACATGGTAGCAATAACAGAATGAAAATCACATTTGGTATGGGGGCTTCACAATATTCGCA
TGACTGTCTGGAAGTAGACCATGCTATTTTTCTGCTCACTGTACACAAATATTGTTCACA
TAAACCCTATAATGTAAATATGAAATACAGTGATTACTCTTCTCACAGGCTGAGTGTATG
AATTCTAAAGACCCATAAGTATTAAAGTGGTAGGGATAAATTGG

SEQ ID NO. 7: U37222_T3 | LENGTH: 1209

ATGAGACCTGGCCACTTTCTCCTCATTTCTGTCTGTACGATTGTCAGTGGATCTGACGAC
ACCAAAAGGGCTCAGGATGCTACTGTTGCAAGCTCTCCTGTTCCTCTTAATCCTGCCCAG
TCATGCCGAAGATGACGTTACTACAACTGAAGAGCTAGCTCCTGCTTTGGTCCCTCCACC
CAAGGGAACTTGTGCAGGTTGGATGGCAGGCATCCCAGGACATCCTGGCCACAATGGCAC
ACCAGGCCGTGATGGCAGAGATGGCACTCCTGGAGAGAAGGGAGAGAAAGGAGATGCAGG
TCTTCTTGGTCCTAAGGGTGAGACAGGAGATGTTGGAATGACAGGAGCTGAAGGGCCACG
GGGCTTCCCCGGAACCCCTGGCAGGAAAGGAGAGCCTGGAGAAGCCGCGTCACTGTTCCC
AATGTACCCATTCGCTTTACTAAGATCTTCTACAACCAACAGAATCATTATGACGGCAGC
ACTGGCAAGTTCTACTGCAACATTCCGGGACTCTACTACTTCTCTTACCACATCACGGTG
TACATGAAAGATGTGAAGGTGAGCCTCTTCAAGAAGGACAAGGCCGTTCTCTTCACCTAC
GACCAGTATCAGGAAAAGAATGTGGACCAGGCCTCTGGCTCTGTGCTCCTCCATCTGGAG
GTGGGAGACCAAGTCTGGCTCCAGGTGTATGGGGATGGGGACCACAATGGACTCTATGCA
GATAACGTCAACGACTCTACATTTACTGGCTTTCTTCTCTACCATGATACCAACTGACTG
CAACTACCCATAGCCCATACACCAGGAGAATCATGGAACAGTCGACACACTTTCAGCTTA
GTTTGAGAGATTGATTTTATTGCTTAGTTTGAGAGTCCTGAGTATTATCCACACGTGTAC
TCACTTGTTCATTAAACGACTTTATAAAAAATAATTTGTGTTCCTAGTCCAGAAAAAAAG
GCACTCCCTGGTCTCCACGACTCTTACATGGTAGCAATAACAGAATGAAAATCACATTTG
GTATGGGGGCTTCACAATATTCGCATGACTGTCTGGAAGTAGACCATGCTATTTTTCTGC
TCACTGTACACAAATATTGTTCACATAAACCCTATAATGTAAATATGAAATACAGTGATT
ACTCTTCTCACAGGCTGAGTGTATGAATTCTAAAGACCCATAAGTATTAAAGTGGTAGGG

ATAAATTGG

SEQ ID NO. 8: U37222_T4 | LENGTH: 1028

ATGAGACCTGGCCACTTTCTCCTCATTTCTGTCTGTACGATTGTCAGTGGATCTGACGAC
ACCAAAAGGGCTCAGGATGCTACTGTTGCAAGCTCTCCTGTTCCTCTTAATCCTGCCCAG
TCATGCCGAAGATGACGTTACTACAACTGAAGAGCTAGCTCCTGCTTTGGTCCCTCCACC
CAAGGGAACTTGTGCAGGTTGGATGGCAGGCATCCCAGGACATCCTGGCCACAATGGCAC
ACCAGGCCGTGATGGCAGAGATGGCACTCCTGGAGAGAAGGGAGAGAAAGGAGATGCAGG
TCTTCTTGGTCCTAAGGGTGAGACAGGAGATGTTGGAATGACAGGAGCTGAAGGGCCACG
GGGCTTCCCCGGAACCCCTGGCAGGAAAGGAGAGCCTGGAGAAGCCGCTTATGTGTATCG
CTCAGCGTTCAGTGTGGGGCTGGAGACCCGCGTCACTGTTCCCAATGTACCCATTCGCTT
TACTAAGATCTTCTACAACCAACAGAATCATTATGACGGCAGCACTGGCAAGTTCTACTG
CAACATTCCGGGACTCTACATTTACTGGCTTTCTTCTCTACCATGATACCAACTGACTGC
AACTACCCATAGCCCATACACCAGGAGAATCATGGAACAGTCGACACACTTTCAGCTTAG
TTTGAGAGATTGATTTTATTGCTTAGTTTGAGAGTCCTGAGTATTATCCACACGTGTACT
CACTTGTTCATTAAACGACTTTATAAAAAATAATTTGTGTTCCTAGTCCAGAAAAAAAGG
CACTCCCTGGTCTCCACGACTCTTACATGGTAGCAATAACAGAATGAAAATCACATTTGG
TATGGGGGCTTCACAATATTCGCATGACTGTCTGGAAGTAGACCATGCTATTTTTCTGCT
CACTGTACACAAATATTGTTCACATAAACCCTATAATGTAAATATGAAATACAGTGATTA
CTCTTCTCACAGGCTGAGTGTATGAATTCTAAAGACCCATAAGTATTAAAGTGGTAGGGA
TAAATTGG

SEQ ID NO. 9: U37222_T5 | LENGTH: 306

ATGAGACCTGGCCACTTTCTCCTCATTTCTGTCTGTACGATTGTCAGTGGATCTGACGAC
ACCAAAAGGGCTCAGGATGCTACTGTTGCAAGCTCTCCTGTTCCTCTTAATCCTGCCCAG
TCATGCCGAAGATGACGTTACTACAACTGAAGAGCTAGCTCCTGCTTTGGTCCCTCCACC
CAAGGGAACTTGTGCAGGTTGGATGGCAGGCATCCCAGGACATCCTGGCCACATAAAAAT
ATAATTCGAGGGGCATCCACCAGGCCGGCTGAATTGTGCCAAAATATGGCACTTCCTGCA
AGATAA

SEQ ID 10-11 Ghrelin variants:

SEQ ID NO. 10: NM_016362_T1 | Length: 665

ACTCTGGATGGGTGCTGTTTAGACAAACGCCGTCTCCTATATAAGACCTGACAGCACAGG
CACCACTCCGCCAGGACTGCAGGCCCACCTGTCTGCAACCCAGCTGAGGCCATGCCCTCC
CCAGGGACCGTCTGCAGCCTCCTGCTCCTCGGCATGCTCTGGCTGGACTTGGCCATGGCA
GGCTCCAGCTTCCTGAGCCCTGAACACCAGAGAGTCCAGCAGAGAAAGGAGTCGAAGAAG
CCACCAGCCAAGCTGCAGCCCCGAGCTCTAGCAGGCTGGCTCCGCCCGGAAGATGGAGGT
CAAGCAGAAGGGGCAGAGGATGAACTGGAAGTCCGGTTCAACGCCCCCTTTGATGTTGGA
ATCAAGCTGTCAGGGGTTCAGTACCAGCAGCACAGCCAGGCCCTGGGGAAGTTTCTTCAG
GACATCCTCTGGGAAGAGGCCAAAGAGGCCCCAGCCGACAAGTGATCGCCCACAAGCCTT
ACTCACCTCTCTCTAAGTTTAGAAGCGCTCATCTGGCTTTTCGCTTGCTTCTGCAGCAAC
TCCCACGACTGTTGTACAAGCTCAGGAGGCGAATAAATGTTCAAACTGTATGCTGATGTT
CCAAATGGGAATTTATTTCAAAGAGGAAAAGTTAATATTTTACTTTAAAAAAATCAAAAT
AATAC

SEQ ID NO. 11: NM_016362_T2 | Length: 579

ACTCTGGATGGGTGCTGTTTAGACAAACGCCGTCTCCTATATAAGACCTGACAGCACAGG

CACCACTCCGCCAGGACTGCAGGCCCACCTGTCTGCAACCCAGCTGAGGCCATGCCCTCC
CCAGGGACCGTCTGCAGCCTCCTGCTCCTCGGCATGCTCTGGCTGGACTTGGCCATGGCA
GGCTCCAGCTTCCTGAGCCCTGAACACCAGAGAGTCCAGGTGAGACCTCCCCACAAAGCC
CCACATGTTGTTCCAGCCCTGCCACTTAGCAACCAGCTCTGTGACCTGGAGCAGCAGCGC
CATCTCTGGGCTTCAGTCTTCTCCCAGAGCACAAAGGACTCTGGGTCTGACCTCACTGTT
TCTGGAAGGACATGGGGGCTTAGAGTCCTAAACAGACTGTTTCCCCCTTCCAGCAGAGAA
AGGAGTCGAAGAAGCCACCAGCCAAGCTGCAGCCCCGAGCTCTAGCAGGCTGGCTCCGCC
CGGAAGATGGAGGTCAAGCAGAAGGGGCAGAGGATGAACTGGAAGTCCGGGTCGGTACCT
CTGCAGTTTTATGCTTCTGTGGCAGCGAGGAGGGTGGGG

SEQ ID 12-21 HSD11B variants:

SEQ ID 12: NM_005525_T1 WT| Length: 1448
GCACTGCCTGAGACTACTCCAGCCTCCCCCGTCCCTGATGTCACAATTCAGAGGCTGCTG
CCTGCTTAGGAGGTTGTAGAAAGCTCTGTAGGTTCTCTCTGTGTGTCCTACAGGAGTCTT
CAGGCCAGCTCCCTGTCGGATGGCTTTTATGAAAAAATATCTCCTCCCCATTCTGGGGCT
CTTCATGGCCTACTACTACTATTCTGCAAACGAGGAATTCAGACCAGAGATGCTCCAAGG
AAAGAAAGTGATTGTCACAGGGGCCAGCAAAGGGATCGGAAGAGAGATGGCTTATCATCT
GGCGAAGATGGGAGCCCATGTGGTGGTGACAGCGAGGTCAAAAGAAACTCTACAGAAGGT
GGTATCCCACTGCCTGGAGCTTGGAGCAGCCTCAGCACACTACATTGCTGGCACCATGGA
AGACATGACCTTCGCAGAGCAATTTGTTGCCCAAGCAGGAAAGCTCATGGGAGGACTAGA
CATGCTCATTCTCAACCACATCACCAACACTTCTTTGAATCTTTTTCATGATGATATTCA
CCATGTGCGCAAAAGCATGGAAGTCAACTTCCTCAGTTACGTGGTCCTGACTGTAGCTGC
CTTGCCCATGCTGAAGCAGAGCAATGGAAGCATTGTTGTCGTCTCCTCTCTGGCTGGGAA
AGTGGCTTATCCAATGGTTGCTGCCTATTCTGCAAGCAAGTTTGCTTTGGATGGGTTCTT
CTCCTCCATCAGAAAGGAATATTCAGTGTCCAGGGTCAATGTATCAATCACTCTCTGTGT
TCTTGGCCTCATAGACACAGAAACAGCCATGAAGGCAGTTTCTGGGATAGTCCATATGCA
AGCAGCTCCAAAGGAGGAATGTGCCCTGGAGATCATCAAAGGGGGAGCTCTGCGCCAAGA
AGAAGTGTATTATGACAGCTCACTCTGGACCACTCTTCTGATCAGAAATCCATGCAGGAA
GATCCTGGAATTTCTCTACTCAACGAGCTATAATATGGACAGATTCATAAACAAGTAGGA
ACTCCCTGAGGGCTGGGCATGCTGAGGGATTTTGGGACTGTTCTGTCTCATGTTTATCTG
AGCTCTTATCTATGAAGACATCTTCCCAGAGTGTCCCCAGAGACATGCAAGTCATGGGTC
ACACCTGACAAATGGAAGGAGTTCCTCTAACATTTGCAAAATGGAAATGTAATAATAATG
AATGTCATGCACCGCTGCAGCCAGCAGTTGTAAAATTGTTAGTAAACATAGGTATAATTA
CCAGATAGTTATATTAAATTTATATCTTATATATAATAATATGTGATGATTAATACAATA
TTAATTATAATAAAGGTCACATAAACTTTATAAATTCATAACTGGTAGCTATAACTTGAG
CTTATTCAGGATGGTTTCTTTAAAACCATAAACTGTACAAATGAAATTTTTCAATATTTG
TTTCTTAT

SEQ ID 13: NM_005525_T2 | LENGTH: 708
GCACTGCCTGAGACTACTCCAGCCTCCCCCGTCCCTGATGTCACAATTCAGAGGCTGCTG
CCTGCTTAGGAGGTTGTAGAAAGCTCTGTAGGTTCTCTCTGTGTGTCCTACAGGAGTCTT
CAGGCCAGCTCCCTGTCGGATGGCTTTTATGAAAAAATATCTCCTCCCCATTCTGGGGCT
CTTCATGGCCTACTACTACTATTCTGCAAACGAGGAATTCAGACCAGAGATGCTCCAAGG
AAAGAAAGTGATTGTCACAGGGGCCAGCAAAGGGATCGGAAGAGAGATGGCTTATCATCT
GGCGAAGATGGGAGCCCATGTGGTGGTGACAGCGAGCTCAGCACACTACATTGCTGGCAC

CATGGAAGACATGACCTTCGCAGAGCAATTTGTTGCCCAAGCAGGAAAGCTCATGGGAGG
ACTAGACATGCTCATTCTCAACCACATCACCAACACTTCTTTGAATCTTTTTCATGATGA
TATTCACCATGTGCGCAAAAGCATGGAAGTCAACTTCCTCAGTTACGTGGTCCTGACTGT
AGCTGCCTTGCCCATGCTGAAGCAGAGCAATGGAAGCATGTGCGCTCTTCTGCTGGAATG
CTATCATGTTGTGCATCTGAGCAGTNGTTGATGGTCTCTCTCATAGAAGATATCAGGCAG
GCATGATATACTTTGGTCTGCTATACCAGACGCTAGGCGTCTGATGCA

SEQ ID 14: NM_005525_T3 | LENGTH: 1394

GCACTGCCTGAGACTACTCCAGCCTCCCCCGTCCCTGATGTCACAATTCAGAGGCTGCTG
CCTGCTTAGGAGGTTGTAGAAAGCTCTGTAGGTTCTCTCTGTGTGTCCTACAGGAGTCTT
CAGGCCAGCTCCCTGTCGGATGGCTTTTATGAAAAAATATCTCCTCCCCATTCTGGGGCT
CTTCATGGCCTACTACTACTATTCTGCAAACGAGGAATTCAGACCAGAGATGCTCCAAGG
AAAGAAAGTGATTGTCACAGGGGCCAGCAAAGGGATCGGAAGAGAGATGGCTTATCATCT
GGCGAAGATGGGAGCCCATGTGGTGGTGACAGCGAGGTCAAAAGAAACTCTACAGAAGGT
GGTATCCCACTGCCTGGAGCTTGGAGCAGCCTCAGCACACTACATTGCTGGCACCATGGA
AGACATGACCTTCGCAGAGCAATTTGTTGCCCAAGCAGGAAAGCTCATGGGAGGACTAGA
CATGCTCATTCTCAACCACATCACCAACACTTCTTTGAATCTTTTTCATGATGATATTCA
CCATGTGCGCAAAAGCATGGAAGTCAACTTCCTCAGTTACGTGGTCCTGACTGTAGCTGC
CTTGCCCATGCTGAAGCAGAGCAATGGAAGCATTGTTGTCGTCTCCTCTCTGGCTGGGAA
AGTGGCTTATCCAATGGTTGCTGCCTATTCTGCAAGCAAGTTTGCTTTGGATGGGTTCTT
CTCCTCCATCAGAAAGGAATATTCAGTGTCCAGGGTCAATGTATCAATCACTCTCTGTGT
TCTTGGCCTCATAGACACAGAAACAGCCATGAAGGCAGTTTCTGGGATAGTCCATATGCA
AGCAGCTCCAAAGGAGGAATGTGCCCTGGAGATCATCAAAGGGGGAGCTCTGCGCCAAGA
AGAAGTGTATTATGACAGCTCACTCTGGACCACTCTTCTGATCAGAAATCCATGCAGGAA
GATCCTGGAATTTCTCTACTCAACGAGCTATAATATGGAGGGACTGTTCTGTCTCATGTT
TATCTGAGCTCTTATCTATGAAGACATCTTCCCAGAGTGTCCCCAGAGACATGCAAGTCA
TGGGTCACACCTGACAAATGGAAGGAGTTCCTCTAACATTTGCAAAATGGAAATGTAATA
ATAATGAATGTCATGCACCGCTGCAGCCAGCAGTTGTAAAATTGTTAGTAAACATAGGTA
TAATTACCAGATAGTTATATTAAATTTATATCTTATATATAATAATATGTGATGATTAAT
ACAATATTAATTATAATAAAGGTCACATAAACTTTATAAATTCATAACTGGTAGCTATAA
CTTGAGCTTATTCAGGATGGTTTCTTTAAAACCATAAACTGTACAAATGAAATTTTTCAA
TATTTGTTTCTTAT

SEQ ID 15: NM_005525_T4 | LENGTH: 1394

GCACTGCCTGAGACTACTCCAGCCTCCCCCGTCCCTGATGTCACAATTCAGAGGCTGCTG
CCTGCTTAGGAGGTTGTAGAAAGCTCTGTAGGTTCTCTCTGTGTGTCCTACAGGAGTCTT
CAGGCCAGCTCCCTGTCGGATGGCTTTTATGAAAAAATATCTCCTCCCCATTCTGGGGCT
CTTCATGGCCTACTACTACTATTCTGCAAACGAGGAATTCAGACCAGAGATGCTCCAAGG
AAAGAAAGTGATTGTCACAGGGGCCAGCAAAGGGATCGGAAGAGAGATGGCTTATCATCT
GGCGAAGATGGGAGCCCATGTGGTGGTGACAGCGAGGTCAAAAGAAACTCTACAGAAGGT
GGTATCCCACTGCCTGGAGCTTGGAGCAGCCTCAGCACACTACATTGCTGGCACCATGGA
AGACATGACCTTCGCAGAGCAATTTGTTGCCCAAGCAGGAAAGCTCATGGGAGGACTAGA
CATGCTCATTCTCAACCACATCACCAACACTTCTTTGAATCTTTTTCATGATGATATTCA
CCATGTGCGCCCCATGCTGAAGCAGAGCAATGGAAGCATTGTTGTCGTCTCCTCTCTGGC
TGGGAAAGTGGCTTATCCAATGGTTGCTGCCTATTCTGCAAGCAAGTTTGCTTTGGATGG

GTTCTTCTCCTCCATCAGAAAGGAATATTCAGTGTCCAGGGTCAATGTATCAATCACTCT
CTGTGTTCTTGGCCTCATAGACACAGAAACAGCCATGAAGGCAGTTTCTGGGATAGTCCA
TATGCAAGCAGCTCCAAAGGAGGAATGTGCCCTGGAGATCATCAAAGGGGGAGCTCTGCG
CCAAGAAGAAGTGTATTATGACAGCTCACTCTGGACCACTCTTCTGATCAGAAATCCATG
CAGGAAGATCCTGGAATTTCTCTACTCAACGAGCTATAATATGGACAGATTCATAAACAA
GTAGGAACTCCCTGAGGGCTGGGCATGCTGAGGGATTTTGGGACTGTTCTGTCTCATGTT
TATCTGAGCTCTTATCTATGAAGACATCTTCCCAGAGTGTCCCCAGAGACATGCAAGTCA
TGGGTCACACCTGACAAATGGAAGGAGTTCCTCTAACATTTGCAAAATGGAAATGTAATA
ATAATGAATGTCATGCACCGCTGCAGCCAGCAGTTGTAAAATTGTTAGTAAACATAGGTA
TAATTACCAGATAGTTATATTAAATTTATATCTTATATATAATAATATGTGATGATTAAT
ACAATATTAATTATAATAAAGGTCACATAAACTTTATAAATTCATAACTGGTAGCTATAA
CTTGAGCTTATTCAGGATGGTTTCTTTAAAACCATAAACTGTACAAATGAAATTTTTCAA
TATTTGTTTCTTAT

SEQ ID 16: NM_005525_T5 | LENGTH: 1394

GCACTGCCTGAGACTACTCCAGCCTCCCCCGTCCCTGATGTCACAATTCAGAGGCTGCTG
CCTGCTTAGGAGGTTGTAGAAAGCTCTGTAGGTTCTCTCTGTGTGTCCTACAGGAGTCTT
CAGGCCAGCTCCCTGTCGGATGGCTTTTATGAAAAAATATCTCCTCCCCATTCTGGGGCT
CTTCATGGCCTACTACTACTATTCTGCAAACGAGGAATTCAGACCAGAGATGCTCCAAGG
AAAGAAAGTGATTGTCACAGGGGCCAGCAAAGGGATCGGAAGAGAGATGGCTTATCATCT
GGCGAAGATGGGAGCCCATGTGGTGGTGACAGCGAGCTCAGCACACTACATTGCTGGCAC
CATGGAAGACATGACCTTCGCAGAGCAATTTGTTGCCCAAGCAGGAAAGCTCATGGGAGG
ACTAGACATGCTCATTCTCAACCACATCACCAACACTTCTTTGAATCTTTTTCATGATGA
TATTCACCATGTGCGCAAAAGCATGGAAGTCAACTTCCTCAGTTACGTGGTCCTGACTGT
AGCTGCCTTGCCCATGCTGAAGCAGAGCAATGGAAGCATTGTTGTCGTCTCCTCTCTGGC
TGGGAAAGTGGCTTATCCAATGGTTGCTGCCTATTCTGCAAGCAAGTTTGCTTTGGATGG
GTTCTTCTCCTCCATCAGAAAGGAATATTCAGTGTCCAGGGTCAATGTATCAATCACTCT
CTGTGTTCTTGGCCTCATAGACACAGAAACAGCCATGAAGGCAGTTTCTGGGATAGTCCA
TATGCAAGCAGCTCCAAAGGAGGAATGTGCCCTGGAGATCATCAAAGGGGGAGCTCTGCG
CCAAGAAGAAGTGTATTATGACAGCTCACTCTGGACCACTCTTCTGATCAGAAATCCATG
CAGGAAGATCCTGGAATTTCTCTACTCAACGAGCTATAATATGGACAGATTCATAAACAA
GTAGGAACTCCCTGAGGGCTGGGCATGCTGAGGGATTTTGGGACTGTTCTGTCTCATGTT
TATCTGAGCTCTTATCTATGAAGACATCTTCCCAGAGTGTCCCCAGAGACATGCAAGTCA
TGGGTCACACCTGACAAATGGAAGGAGTTCCTCTAACATTTGCAAAATGGAAATGTAATA
ATAATGAATGTCATGCACCGCTGCAGCCAGCAGTTGTAAAATTGTTAGTAAACATAGGTA
TAATTACCAGATAGTTATATTAAATTTATATCTTATATATAATAATATGTGATGATTAAT
ACAATATTAATTATAATAAAGGTCACATAAACTTTATAAATTCATAACTGGTAGCTATAA
CTTGAGCTTATTCAGGATGGTTTCTTTAAAACCATAAACTGTACAAATGAAATTTTTCAA
TATTTGTTTCTTAT

SEQ ID 17: NM_005525_T6 | LENGTH: 1821

GGTGAAAAGGGAAAACCTGCCCAAATCCAGTTTTTGTTTCAGTAACTTCCTTTGAGACAA
AGTCAGGAATCTGAGAGTAAGCACCTGCTAAGGGTGGGACAGGGGCTCTGTCTGGTATGC
CTCTCCCATGTTAAGAGCTAACAATAGTAATGGATAAGTCTCCAGGGCAACCAGGACCAC
TTCCAAGCATTCCTGTCTTGGGCTGCCTCGAGGGCTCCTCTGTCCTTTGGGGAGTACTGA

TTGATGCCTGATGCCCAGAACTGGCCCACTCTGGCTTCTCTTTGGAGCTGTCTCTGCAGG
CGCCTTCTGGCTGCCAGCTCGGTCCTAGCATAAGGGACTTCTTCCTTGGCCTGGGTTTCA
CCTTCTTGTATCAGGTGGCAGACCAGCTGGTTTCAGTCCCAAATCAGGTCTTCTGACTCC
TCCCAGAAACCAACCAACTTCTGAGCAGGAAATCCTGCCCCTCCCCAAAGAGTGGGAAAC
CGCAAAGGAAGAGAGAGATGAAACAGAAGGAAAGGCAGAGGAGGAGGGAGAGAGAGAAA
GAGAAGAAAAAGAAAAAAGAACATCAATAAAAAGAAGTCAGATTTGTTCGAAATCTTGAG
AGATGCTCCAAGGAAAGAAAGTGATTGTCACAGGGGCCAGCAAAGGGATCGGAAGAGAGA
TGGCTTATCATCTGGCGAAGATGGGAGCCCATGTGGTGGTGACAGCGAGGTCAAAAGAAA
CTCTACAGAAGGTGGTATCCCACTGCCTGGAGCTTGGAGCAGCCTCAGCACACTACATTG
CTGGCACCATGGAAGACATGACCTTCGCAGAGCAATTTGTTGCCCAAGCAGGAAAGCTCA
TGGGAGGACTAGACATGCTCATTCTCAACCACATCACCAACACTTCTTTGAATCTTTTTC
ATGATGATATTCACCATGTGCGCAAAAGCATGGAAGTCAACTTCCTCAGTTACGTGGTCC
TGACTGTAGCTGCCTTGCCCATGCTGAAGCAGAGCAATGGAAGCATTGTTGTCGTCTCCT
CTCTGGCTGGGAAAGTGGCTTATCCAATGGTTGCTGCCTATTCTGCAAGCAAGTTTGCTT
TGGATGGGTTCTTCTCCTCCATCAGAAAGGAATATTCAGTGTCCAGGGTCAATGTATCAA
TCACTCTCTGTGTTCTTGGCCTCATAGACACAGAAACAGCCATGAAGGCAGTTTCTGGGA
TAGTCCATATGCAAGCAGCTCCAAAGGAGGAATGTGCCCTGGAGATCATCAAAGGGGGAG
CTCTGCGCCAAGAAGAAGTGTATTATGACAGCTCACTCTGGACCACTCTTCTGATCAGAA
ATCCATGCAGGAAGATCCTGGAATTTCTCTACTCAACGAGCTATAATATGGACAGATTCA
TAAACAAGTAGGAACTCCCTGAGGGCTGGGCATGCTGAGGGATTTTGGGACTGTTCTGTC
TCATGTTTATCTGAGCTCTTATCTATGAAGACATCTTCCCAGAGTGTCCCCAGAGACATG
CAAGTCATGGGTCACACCTGACAAATGGAAGGAGTTCCTCTAACATTTGCAAAATGGAAA
TGTAATAATAATGAATGTCATGCACCGCTGCAGCCAGCAGTTGTAAAATTGTTAGTAAAC
ATAGGTATAATTACCAGATAGTTATATTAAATTTATATCTTATATATAATAATATGTGAT
GATTAATACAATATTAATTATAATAAAGGTCACATAAACTTTATAAATTCATAACTGGTA
GCTATAACTTGAGCTTATTCAGGATGGTTTCTTTAAAACCATAAACTGTACAAATGAAAT
TTTTCAATATTTGTTTCTTAT

SEQ ID 18: NM_005525_T7 | LENGTH: 1304

GCACTGCCTGAGACTACTCCAGCCTCCCCCGTCCCTGATGTCACAATTCAGAGGCTGCTG
CCTGCTTAGGAGGTTGTAGAAAGCTCTGTAGGTTCTCTCTGTGTGTCCTACAGGAGTCTT
CAGGCCAGCTCCCTGTCGGATGGCTTTTATGAAAAAATATCTCCTCCCCATTCTGGGGCT
CTTCATGGCCTACTACTACTATTCTGCAAACGAGGAATTCAGACCAGAGATGCTCCAAGG
AAAGAAAGTGATTGTCACAGGGGCCAGCAAAGGGATCGGAAGAGAGATGGCTTATCATCT
GGCGAAGATGGGAGCCCATGTGGTGGTGACAGCGAGGTCAAAAGAAACTCTACAGAAGGT
GGTATCCCACTGCCTGGAGCTTGGAGCAGCCTCAGCACACTACATTGCTGGCACCATGGA
AGACATGACCTTCGCAGAGCAATTTGTTGCCCAAGCAGGAAAGCTCATGGGAGGACTAGA
CATGCTCATTCTCAACCACATCACCAACACTTCTTTGAATCTTTTTCATGATGATATTCA
CCATGTGCGCAAAAGCATGGAAGTCAACTTCCTCAGTTACGTGGTCCTGACTGTAGCTGC
CTTGCCCATGCTGAAGCAGAGCAATGGAAGCATTGTTGTCGTCTCCTCTCTGGCTGAAAC
AGCCATGAAGGCAGTTTCTGGGATAGTCCATATGCAAGCAGCTCCAAAGGAGGAATGTGC
CCTGGAGATCATCAAAGGGGGAGCTCTGCGCCAAGAAGAAGTGTATTATGACAGCTCACT
CTGGACCACTCTTCTGATCAGAAATCCATGCAGGAAGATCCTGGAATTTCTCTACTCAAC
GAGCTATAATATGGACAGATTCATAAACAAGTAGGAACTCCCTGAGGGCTGGGCATGCTG

AGGGATTTTGGGACTGTTCTGTCTCATGTTTATCTGAGCTCTTATCTATGAAGACATCTT
CCCAGAGTGTCCCCAGAGACATGCAAGTCATGGGTCACACCTGACAAATGGAAGGAGTTC
CTCTAACATTTGCAAAATGGAAATGTAATAATAATGAATGTCATGCACCGCTGCAGCCAG
CAGTTGTAAAATTGTTAGTAAACATAGGTATAATTACCAGATAGTTATATTAAATTTATA
TCTTATATATAATAATATGTCATGATTAATACAATATTAATTATAATAAAGGTCACATAA
ACTTTATAAATTCATAACTGGTAGCTATAACTTGAGCTTATTCAGGATGGTTTCTTTAAA
ACCATAAACTGTACAAATGAAATTTTTCAATATTTGTTTCTTAT

SEQ ID 19: XM_110304_T1 | Length: 1307 WT

ACTGTTGGCCTCTGGAWTCAGAGGCTGCTGCCTGCCTGGGAGGTTGTAGAAAGCTCTGCA
GGTTTTCTTCGTGTGTCCTACAGGGCGCCCTGAGCCAGGTCCCTGTTTGATGGCAGTTAT
GAAAAATTACCTCCTCCCGATCCTGGTGCTCTTCCTGGCCTACTACTACTATTCTACAAA
TGAAGAGTTCAGACCAGAAATGCTCCAGGGAAAGAAAGTGATTGTCACTGGGGCCAGCAA
AGGGATTGGAAGAGAAATGGCATATCATCTGTCAAAAATGGGAGCCCATGTGGTATTGAC
TGCCAGGTCGGAGGAAGGTCTCCAGAAGGTAGTGTCTCGCTGCCTTGAACTCGGAGCAGC
CTCTGCTCACTACATTGCTGGCACTATGGAAGACATGACATTTGCGGAGCAATTTATTGT
CAAGGCGGGAAAGCTCATGGGCGGACTGGACATGCTTATTCTAAACCACATCACTCAGAC
CTCGCTGTCTCTCTTCCATGACGACATCCACTCTGTGCGAAGAGTCATGGAGGTCAACTT
CCTCAGCTACGTGGTCATGAGCACAGCCGCCTTGCCCATGCTGAAGCAGAGCAATGGCAG
CATTGCCGTCATCTCCTCCTTGGCTGGGAAAATGACCCAGCCTATGATTGCTCCCTACTC
TGCAAGCAAGTTTGCTCTGGATGGGTTCTTTTCCACCATTAGAACAGAACTCTACATAAC
CAAGGTCAACGTGTCCATCACTCTCTGTGTCCTTGGCCTCATAGACACAGAAACAGCTAT
GAAGGAAATCTCTGGGATAATTAACGCCCAAGCTTCTCCCAAGGAGGAGTGCGCCCTGGA
GATCATCAAAGGCACAGCTCTACGCAAAAGCGAGGTGTACTATGACAAATCGCCTTTGAC
TCCAATCCTGCTTGGGAACCCAGGAAGGAAGATCATGGAATTTTTTTCATTACGATATTA
TAATAAGGACATGTTTGTAAGTAACTAGGAACTCCTGAGCCCTGGTGAGTGGTCTTAGAA
CAGTCCTGCCTGATACTTCTGTAAGCCCTACCCACAAAAGTATCTTTCCAGAGATACACA
AATTTTGGGGTACACCTCATCATGAGAAATTCTTGCAACACTTGCACAGTGAAAATGTAA
TTGTAATAAATGTCACAAACCACTTTGGGGCCTGCAGTTGTGAACTTGATTGTAACTATG
GATATAAACACATAGTGGTTGTATCGGCTTTACCTCACACTGAATGAAACAATGATAACT
AATGTAACATTAAATATAATAAAGGTAATATCAACTTTGTAAATGCA

SEQ ID 20: XM_110304_T3 | Length: 1181

ACTGTTGGCCTCTGGAWTCAGAGGCTGCTGCCTGCCTGGGAGGTTGTAGAAAGCTCTGCA
GGTTTTCTTCGTGTGTCCTACAGGGCGCCCTGAGCCAGGTCCCTGTTTGATGGCAGTTAT
GAAAAATTACCTCCTCCCGATCCTGGTGCTCTTCCTGGCCTACTACTACTATTCTACAAA
TGAAGAGTTCAGACTCCAGAAGGTAGTGTCTCGCTGCCTTGAACTCGGAGCAGCCTCTGC
TCACTACATTGCTGGCACTATGGAAGACATGACATTTGCGGAGCAATTTATTGTCAAGGC
GGGAAAGCTCATGGGCGGACTGGACATGCTTATTCTAAACCACATCACTCAGACCTCGCT
GTCTCTCTTCCATGACGACATCCACTCTGTGCGAAGAGTCATGGAGGTCAACTTCCTCAG
CTACGTGGTCATGAGCACAGCCGCCTTGCCCATGCTGAAGCAGAGCAATGGCAGCATTGC
CGTCATCTCCTCCTTGGCTGGGAAAATGACCCAGCCTATGATTGCTCCCTACTCTGCAAG
CAAGTTTGCTCTGGATGGGTTCTTTTCCACCATTAGAACAGAACTCTACATAACCAAGGT
CAACGTGTCCATCACTCTCTGTGTCCTTGGCCTCATAGACACAGAAACAGCTATGAAGGA
AATCTCTGGGATAATTAACGCCCAAGCTTCTCCCAAGGAGGAGTGCGCCCTGGAGATCAT

CAAAGGCACAGCTCTACGCAAAAGCGAGGTGTACTATGACAAATCGCCTTTGACTCCAAT
CCTGCTTGGGAACCCAGGAAGGAAGATCATGGAATTTTTTTCATTACGATATTATAATAA
GGACATGTTTGTAAGTAACTAGGAACTCCTGAGCCCTGGTGAGTGGTCTTAGAACAGTCC
TGCCTGATACTTCTGTAAGCCCTACCCACAAAAGTATCTTTCCAGAGATACACAAATTTT
GGGGTACACCTCATCATGAGAAATTCTTGCAACACTTGCACAGTGAAAATGTAATTGTAA
TAAATGTCACAAACCACTTTGGGGCCTGCAGTTGTGAACTTGATTGTAACTATGGATATA
AACACATAGTGGTTGTATCGGCTTTACCTCACACTGAATGAAACAATGATAACTAATGTA
ACATTAAATATAATAAAGGTAATATCAACTTTGTAAATGCA

SEQ ID 21: XM_110304_T4 | Length: 845

ACTGTTGGCCTCTGGAWTCAGAGGCTGCTGCCTGCCTGGGAGGTTGTAGAAAGCTCTGCA
GGTTTTCTTCGTGTGTCCTACAGGGCGCCCTGAGCCAGGTCCCTGTTTGATGGCAGTTAT
GAAAAATTACCTCCTCCCGATCCTGGTGCTCTTCCTGGCCTACTACTACTATTCTACAAA
TGAAGAGTTCAGACCAGAAATGCTCCAGGGAAAGAAAGTGATTGTCACTGGGGCCAGCAA
AGGGATTGGAAGAGAAATGGCATATCATCTGTCAAAAATGGGAGCCCATGTGGTATTGAC
TGCCAGGTCGGAGGAAGGTCTCCAGAAGGTAGTGTCTCGCTGCCTTGAACTCGGAGCAGC
CTCTGCTCACTACATTGCTGGCACTATGGAAGACATGACATTTGCGGAGCAATTTATTGT
CAAGGCGGGAAAGCTCATGGGCGGACTGGACATGCTTATTCTAAACCACATCACTCAGAC
CTCGCTGTCTCTCTTCCATGACGACATCCACTCTGTGCGAAGAGTCATGGAGGTCAACTT
CCTCAGCTACGTGGTCATGAGCACAGCCGCCTTGCCCATGCTGAAGCAGAGCAATGGCAG
CATTGCCGTCATCTCCTCCTTGGCTGGGGGAAGAACAGTTCCACAACAGAGAAGTCGCAG
TGTTACTCCTGACTCCCGCGGCCCGTGATTAATATCACCAGCCACAGAATGGACTGGAAC
CCTGTATCGATCTGGTGGGATTGGATATAACGAACATAGAATTACTCCTGAGACTACCAG
AACTGAATAGTTCAAATCAAATCATGCCAGAATATCAGACAAATCCAAATGGCAAAACAG
TTGCA

SEQ ID 22-30 Adiponectin variants products:

SEQ ID NO. 22: NP_004788_P1 | Length: 244 | Transcript: 1 WT

MLLLGAVLLLLALPGHDQETTTQGPGVLLPLPKGACTGWMAGIPGHPGHNGAPGRDGRDG
TPGEKGEKGDPGLIGPKGDIGETGVPGAEGPRGFPGIQGRKGEPGEGAYVYRSAFSVGLE
TYVTIPNMPIRFTKIFYNQQNHYDGSTGKFHCNIPGLYYFAYHITVYMKDVKVSLFKKDK
AMLFTYDQYQENNVDQASGSVLLHLEVGDQVWLQVYGEGERNGLYADNDNDSTFTGFLLY
HDTN

SEQ ID NO. 23: NP_004788_P2 | Length: 160 | Transcript: 2

MPGAEGPRGFPGIQGRKGEPGEGAYVYRSAFSVGLETYVTIPNMPIRFTKIFYNQQNHYD
GSTGKFHCNIPGLYYFAYHITVYMKDVKVSLFKKDKAMLFTYDQYQENNVDQASGSVLLH
LEVGDQVWLQVYGEGERNGLYADNDNDSTFTGFLLYHDTN

SEQ ID NO. 24: NP_004788_P3 | Length: 153 | Transcript: 3

MLLLGAVLLLLALPGHDQETTTQGPGVLLPLPKGACTGWMAGIPGHPGHNGAPGRDGRDG
TPGEKGEKGDPGLIGPKGDIGETGVPGAEGPRGFPGIQGRKGEPGEGALLSPTCPFALPR
SSTISKTTMMAPLVNSTATFLGCTTLPTTSQSI

SEQ ID NO. 25: NP_004788_P4 | Length: 166 | Transcript: 4

MLLLGAVLLLLALPGHDQETTTQGPGVLLPLPKGACTGWMAGIPGHPGHNGAPGRDGRDG
TPGEKGEKGDPGLIGPKGDIGETGVPGAEGPRGFPGIQGRKGEPGEGAYVYRSAFSVGLE

TYVTIPNMPIRFTKIFYNQQNHYDGSTGKFHCNIPGLYLHRLSSLP

SEQ ID NO. 26: NP_033735_P1 | Length: 247 | Transcript: 1 WT

MLLLQALLFLLILPSHAEDDVTTTEELAPALVPPPKGTCAGWMAGIPGHPGHNGTPGRDG
RDGTPGEKGEKGDAGLLGPKGETGDVGMTGAEGPRGFPGTPGRKGEPGEAAYMYRSAFSV
GLETRVTVPNVPIRFTKIFYNQQNHYDGSTGKFYCNIPGLYYFSYHITVYMKDVKVSLFK
KDKAVLFTYDQYQEKNVDQASGSVLLHLEVGDQVWLQVYGDGDHNGLYADNVNDSTFTGF
LLYHDTN

SEQ ID NO. 27: NP_033735_P2 | Length: 160 | Transcript: 2

MTGAEGPRGFPGTPGRKGEPGEAAYVYRSAFSVGLETRVTVPNVPIRFTKIFYNQQNHYD
GSTGKFYCNIPGLYYFSYHITVYMKDVKVSLFKKDKAVLFTYDQYQEKNVDQASGSVLLH
LEVGDQVWLQVYGDGDHNGLYADNVNDSTFTGFLLYHDTN

SEQ ID NO. 28: NP_033735_P3 | Length: 156 | Transcript: 3

MLLLQALLFLLILPSHAEDDVTTTEELAPALVPPPKGTCAGWMAGIPGHPGHNGTPGRDG
RDGTPGEKGEKGDAGLLGPKGETGDVGMTGAEGPRGFPGTPGRKGEPGEAASLFPMYPFA
LLRSSTTNRIIMTAALASSTATFRDSTTSLTTSRCT

SEQ ID NO. 29: NP_033735_P4 | Length: 169 | Transcript: 4

MLLLQALLFLLILPSHAEDDVTTTEELAPALVPPPKGTCAGWMAGIPGHPGHNGTPGRDG
RDGTPGEKGEKGDAGLLGPKGETGDVGMTGAEGPRGFPGTPGRKGEPGEAAYVYRSAFSV
GLETRVTVPNVPIRFTKIFYNQQNHYDGSTGKFYCNIPGLYIYWLSSLP

SEQ ID NO. 30: NP_033735_P5 | Length: 76 | Transcript: 5

MLLLQALLFLLILPSHAEDDVTTTEELAPALVPPPKGTCAGWMAGIPGHPGHIKIKFEGH
PPGRLNCAKIWHFLQD

SEQ ID 31-32 Ghrelin variants :

SEQ ID NO. 31: NP_057446 | Length: 117 | Transcript: 1 WT

MPSPGTVCSLLLLGMLWLDLAMAGSSFLSPEHQRVQQRKESKKPPAKLQPRALAGWLRPE
DGGQAEGAEDELEVRFNAPFDVGIKLSGVQYQQHSQALGKFLQDILWEEAKEAPADK

SEQ ID NO. 32: NP_057446 | Length: 117 | Transcript: 2

MPSPGTVCSLLLLGMLWLDLAMAGSSFLSPEHQRVQVRPPHKAPHVVPALPLSNQLCDLE
QQRHWASVFSQSTKDSGSDLTVSGRTWGLRVLNRLFPPSSRERSRRSHQPSCSPEL

SEQ ID 33-42 HSD11B variants:

SEQ ID NO. 33: NP_005516 | Length: 292 | Transcript: 1 WT

MAFMKKYLLPILGLFMAYYYYSANEEFRPEMLQGKKVIVTGASKGIGREMAYHLAKMGAH
VVVTARSKETLQKVVSHCLELGAASAHYIAGTMEDMTFAEQFVAQAGKLMGGLDMLILNH
ITNTSLNLFHDDIHHVRKSMEVNFLSYVVLTVAALPMLKQSNGSIVVVSSLAGKVAYPMV
AAYSASKFALDGFFSSIRKEYSVSRVNVSITLCVLGLIDTETAMKAVSGIVHMQAAPKEE
CALEIIKGGALRQEEVYYDSSLWTTLLIRNPCRKILEFLYSTSYNMDRFINK

SEQ ID NO. 34: NP_005516 | Length: 163 | Transcript: 2

MAFMKKYLLPILGLFMAYYYYSANEEFRPEMLQGKKVIVTGASKGIGREMAYHLAKMGAH
VVVTASSAHYIAGTMEDMTFAEQFVAQAGKLMGGLDMLILNHITNTSLNLFHDDIHHVRK
SMEVNFLSYVVLTVAALPMLKQSNGSMCALLLECYHVVHLSSX

SEQ ID NO. 35: NP_005516 | Length: 295 | Transcript: 3

MAFMKKYLLPILGLFMAYYYYSANEEFRPEMLQGKKVIVTGASKGIGREMAYHLAKMGAH

VVVTARSKETLQKVVSHCLELGAASAHYIAGTMEDMTFAEQFVAQAGKLMGGLDMLILNH
ITNTSLNLFHDDIHHVRKSMEVNFLSYVVLTVAALPMLKQSNGSIVVVSSLAGKVAYPMV
AAYSASKFALDGFFSSIRKEYSVSRVNVSITLCVLGLIDTETAMKAVSGIVHMQAAPKEE
CALEIIKGGALRQEEVYYDSSLWTTLLIRNPCRKILEFLYSTSYNMEGLFCLMFI

SEQ ID NO. 36: NP_005516 | Length: 274 | Transcript: 4

MAFMKKYLLPILGLFMAYYYYSANEEFRPEMLQGKKVIVTGASKGIGREMAYHLAKMGAH
VVVTARSKETLQKVVSHCLELGAASAHYIAGTMEDMTFAEQFVAQAGKLMGGLDMLILNH
ITNTSLNLFHDDIHHVRPMLKQSNGSIVVVSSLAGKVAYPMVAAYSASKFALDGFFSSIR
KEYSVSRVNVSITLCVLGLIDTETAMKAVSGIVHMQAAPKEECALEIIKGGALRQEEVYY
DSSLWTTLLIRNPCRKILEFLYSTSYNMDRFINK

SEQ ID NO. 37: NP_005516 | Length: 274 | Transcript: 5

MAFMKKYLLPILGLFMAYYYYSANEEFRPEMLQGKKVIVTGASKGIGREMAYHLAKMGAH
VVVTASSAHYIAGTMEDMTFAEQFVAQAGKLMGGLDMLILNHITNTSLNLFHDDIHHVRK
SMEVNFLSYVVLTVAALPMLKQSNGSIVVVSSLAGKVAYPMVAAYSASKFALDGFFSSIR
KEYSVSRVNVSITLCVLGLIDTETAMKAVSGIVHMQAAPKEECALEIIKGGALRQEEVYY
DSSLWTTLLIRNPCRKILEFLYSTSYNMDRFINK

SEQ ID NO. 38: NP_005516 | Length: 262 | Transcript: 6

MLQGKKVIVTGASKGIGREMAYHLAKMGAHVVVTARSKETLQKVVSHCLELGAASAHYIA
GTMEDMTFAEQFVAQAGKLMGGLDMLILNHITNTSLNLFHDDIHHVRKSMEVNFLSYVVL
TVAALPMLKQSNGSIVVVSSLAGKVAYPMVAAYSASKFALDGFFSSIRKEYSVSRVNVSI
TLCVLGLIDTETAMKAVSGIVHMQAAPKEECALEIIKGGALRQEEVYYDSSLWTTLLIRN
PCRKILEFLYSTSYNMDRFINK

SEQ ID NO. 39: NP_005516 | Length: 244 | Transcript: 7

MAFMKKYLLPILGLFMAYYYYSANEEFRPEMLQGKKVIVTGASKGIGREMAYHLAKMGAH
VVVTARSKETLQKVVSHCLELGAASAHYIAGTMEDMTFAEQFVAQAGKLMGGLDMLILNH
ITNTSLNLFHDDIHHVRKSMEVNFLSYVVLTVAALPMLKQSNGSIVVVSSLAETAMKAVS
GIVHMQAAPKEECALEIIKGGALRQEEVYYDSSLWTTLLIRNPCRKILEFLYSTSYNMDR
FINK

SEQ ID NO. 40: XP_110304| Length: 292 | Transcript: 1 WT

MAVMKNYLLPILVLFLAYYYYSTNEEFRPEMLQGKKVIVTGASKGIGREMAYHLSKMGAH
VVLTARSEEGLQKVVSRCLELGAASAHYIAGTMEDMTFAEQFIVKAGKLMGGLDMLILNH
ITQTSLSLFHDDIHSVRRVMEVNFLSYVVMSTAALPMLKQSNGSIAVISSLAGKMTQPMI
APYSASKFALDGFFSTIRTELYITKVNVSITLCVLGLIDTETAMKEISGIINAQASPKEE
CALEIIKGTALRKSEVYYDKSPLTPILLGNPGRKIMEFFSLRYYNKDMFVSN

SEQ ID NO. 41: XP_110304| Length: 250 | Transcript: 8

MAVMKNYLLPILVLFLAYYYYSTNEEFRLQKVVSRCLELGAASAHYIAGTMEDMTFAEQF
IVKAGKLMGGLDMLILNHITQTSLSLFHDDIHSVRRVMEVNFLSYVVMSTAALPMLKQSN
GSIAVISSLAGKMTQPMIAPYSASKFALDGFFSTIRTELYITKVNVSITLCVLGLIDTET
AMKEISGIINAQASPKEECALEIIKGTALRKSEVYYDKSPLTPILLGNPGRKIMEFFSLR
YYNKDMFVSN

SEQ ID NO. 42: XP_110304| Length: 192 | Transcript: 9

MAVMKNYLLPILVLFLAYYYYSTNEEFRPEMLQGKKVIVTGASKGIGREMAYHLSKMGAH
VVLTARSEEGLQKVVSRCLELGAASAHYIAGTMEDMTFAEQFIVKAGKLMGGLDMLILNH

ITQTSLSLFHDDIHSVRRVMEVNFLSYVVMSTAALPMLKQSNGSIAVISSLAGGRTVPQQ
RSRSVTPDSRGP

"obesity and/or diabetes Variants products – also referred at times as the "obesity and/or diabetes variants proteins" or ***"obesity and/or diabetes variants polypeptides"*** – is an amino acid sequence encoded by the obesity and/or diabetes variants nucleic acid sequences which is a naturally occurring mRNA sequence obtained as a result of alternative splicing. The amino acid sequences may be a peptide, a protein, as well as peptides or proteins having *chemically modified* amino acids (see below) such as a glycopeptide or glycoprotein. The obesity and/or diabetes variants products are shown in any one of SEQ ID NO: 22 to SEQ ID NO: 42. The term also includes *homologs* (see below) of said sequences in which one or more amino acids has been added, deleted, *substituted* (see below) or *chemically modified* (see below) as well as *fragments* (see below) of this sequence having at least 10 amino acids.

"Fragments of obesity and/or diabetes related variants nucleic acid sequences" – a partial sequence of any one of SEQ ID NO: 1 to SEQ ID NO: 21 which includes the regions which contains the variation in nucleotides between the variant and the original sequences. These regions (in the amino acid level) are as depicted in the above Table 1.

"Fragments of obesity and/or diabetes related variant product" – amino acid sequences coded by the above nucleic acid fragment, containing regions by which the variant differs from the original sequence as indicated in Table 1.

"Nucleic acid sequence" – a sequence composed of DNA nucleotides, RNA nucleotides or a combination of both types and may includes natural nucleotides, chemically modified nucleotides and synthetic nucleotides.

"Amino acid sequence" – a sequence composed of any one of the 20 naturally appearing amino acids, amino acids which have been *chemically modified* (see below), or composed of synthetic amino acids.

"Homologues of variants/products" – amino acid sequences of variants in which one or more amino acids has been added, deleted or replaced. The altered amino acid shall be in regions where the variant differs from the original sequence, for example, according to the explanation in Table 1.

"Conservative substitution" - refers to the substitution of an amino acid in one class by an amino acid of the same class, where a class is defined by common physicochemical amino acid side chain properties and high substitution frequencies in homologous proteins found in nature, as determined, for example, by a standard Dayhoff frequency exchange matrix or BLOSUM matrix. Six general classes of amino acid side chains have been categorized and include: Class I (Cys); Class II (Ser, Thr, Pro, Ala, Gly); Class III (Asn, Asp, Gln, Glu); Class IV (His, Arg, Lys); Class V (Ile, Leu, Val, Met); and Class VI (Phe, Tyr, Trp). For example, substitution of an Asp for another class III residue such as Asn, Gln, or Glu, is a conservative substitution.

"Non-conservative substitution" - refers to the substitution of an amino acid in one class with an amino acid from another class; for example, substitution of an Ala, a class II residue, with a class III residue such as Asp, Asn, Glu, or Gln.

"Chemically modified" - when referring to the product of the invention, means a product (protein) where at least one of its amino acid resides is modified either by natural processes, such as processing or other post-translational modifications, or by chemical modification techniques which are well known in the art. Among the numerous known modifications typical, but not exclusive examples include: acetylation, acylation, amidation, ADP-ribosylation, glycosylation, GPI anchor formation, covalent attachment of a lipid or lipid derivative, methylation, myristlyation, pegylation, prenylation, phosphorylation, ubiqutination, or any similar process.

"Biologically active" - refers to the variant product having some sort of biological activity, for example, capability of binding to the obesity and/or diabetes related gene or to other agonists of the original obesity and/or diabetes related gene as known.

"Immunologically active" defines the capability of a natural, recombinant or synthetic varient product, or any fragment thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies. Thus, for example, an immunologically active fragment of variant product denotes a fragment which retains some or all of the immunological properties of the variant product, e.g can bind specific anti-variant product antibodies or which can elicit an immune response which will generate such antibodies or cause proliferation of specific immune cells which produce variant.

"Optimal alignment" - is defined as an alignment giving the highest percent identity score. Such alignment can be performed using a variety of commercially available sequence analysis programs, such as the local alignment program LALIGN using a ktup of 1, default parameters and the default PAM. A preferred alignment is the one performed using the CLUSTAL-W program from MacVector

(TM), operated with an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM similarity matrix. If a gap needs to be inserted into a first sequence to optimally align it with a second sequence, the percent identity is calculated using only the residues that are paired with a corresponding amino acid residue (i.e., the calculation does not consider residues in the second sequences that are in the *"gap"* of the first sequence). In case of alignments of known gene sequences with that of the new variant, the optimal alignment invariably included aligning the identical parts of both sequences together, then keeping apart and unaligned the sections of the sequences that differ one from the other.

"Having at least 90% identity" - with respect to two amino acid or nucleic acid sequence sequences, refers to the percentage of residues that are identical in the two sequences when the sequences are optimally aligned. Thus, 90% amino acid sequence identity means that 90% of the amino acids in two or more optimally aligned polypeptide sequences are identical.

"Isolated nucleic acid molecule having an variant nucleic acid sequence" - is a nucleic acid molecule that includes the obesity and/or diabetes related variant nucleic acid coding sequence. Said isolated nucleic acid molecule may include the obesity and/or diabetes related variant nucleic acid sequence as an independent insert; may include the obesity and/or diabetes related variant nucleic acid sequence fused to an additional coding sequences, encoding together a fusion protein in which the variant coding sequence is the dominant coding sequence (for example, the additional coding sequence may code for a signal peptide); the obesity and/or diabetes related variant nucleic acid sequence may be in combination with non-coding sequences, e.g., introns or control elements, such as promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host; or may be a vector in which the obesity and/or diabetes related variant protein coding sequence is a heterologous.

"Expression vector" - refers to vectors that have the ability to incorporate and express heterologous DNA fragments in a foreign cell. Many prokaryotic and eukaryotic expression vectors are known and/or commercially available. Selection of appropriate expression vectors is within the knowledge of those having skill in the art.

"Deletion" - is a change in either nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent.

"Insertion" or "addition" - is that change in a nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring sequence.

"Substitution" - replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively. As regards amino acid sequences the substitution may be conservative or non- conservative.

"Antibody" – refers to IgG, IgM, IgD, IgA, and IgG antibody. The definition includes polyclonal antibodies or monoclonal antibodies. This term refers to whole antibodies or fragments of the antibodies comprising the antigen-binding domain of the anti-variant product antibodies, e.g. antibodies without the Fc portion, single chain antibodies, fragments consisting of essentially only the variable, antigen-binding domain of the antibody, etc.

"Treating a disease" - refers to administering a therapeutic substance effective to ameliorate symptoms associated with a disease, to lessen the severity or cure the disease, or to prevent the disease from occurring.

"***Detection***" – refers to a method of detection of a disease, disorder, pathological or normal condition. This term may refer to detection of a predisposition to a disease as well as for establishing the prognosis of the patient by determining the severity of the disease.

"Probe" – the obesity and/or diabetes variant nucleic acid sequence, or a sequence complementary therewith, when used to detect presence of other similar sequences in a sample or of sequences having some homology with this sequence. The detection is carried out by identification of hybridization complexes between the probe and the assayed sequence. The probe may be attached to a solid support or to a detectable label.

"Original obesity and/or diabetes related genes" – the amino acid or nucleic acid sequence from which the obesity and/or diabetes related variants of the invention have been varied as a result of alternative slicing. The original nucleic sequence is the sequence of the human obesity and/or diabetes related gene depicted as SEQ ID NO: 1 for human Adiponectin and the original amino acid sequence is the sequence encoded by it ; SEQ ID NO: 5 for mouse Adiponectin and the original amino acid sequence is the sequence encoded by it ; SEQ ID NO: 10 for Ghrelin and the original amino acid sequence is the sequence encoded by it ; SEQ ID NO: 12 for human 11-beta-HSD and the original amino acid sequence is the sequence encoded by it ; SEQ ID NO: 19 for mouse 11-beta-HSD and the original amino acid sequence is the sequence encoded by it.

SUMMARY OF THE INVENTION

The present invention relates to isolated nucleic acid molecules having a sequence selected from the group consisting of: SEQ ID NO: 2-4;6-9;11;13-18;20-21 and fragments thereof comprising at least 10 nucleotides. The present invention relates to isolated nucleic acid molecules comprising SEQ ID NO: 2-4;6-9;11;13-18;20-21

and isolated nucleic acid molecules comprising fragments of SEQ ID NO: 2-4;6-9;11;13-18;20-21 comprising at least 10 nucleotides.

The present invention relates to PCR primers which can amplify products using sequences of SEQ ID NO: 2-4;6-9;11;13-18;20-21 as templates.

The present invention relates to methods of screening, diagnosing and monitoring individuals for obesity and/or diabetes. The methods comprise detecting the presence, absence, or quantity of a transcription product that comprises a sequence selected from the group consisting of: SEQ ID NO: 2-4;6-9;11;13-18;20-21 in a sample. The presence or quantity of said transcription product is indicative of obesity and/or diabetes.

The present invention relates to methods of screening, diagnosing and monitoring individuals for obesity and/or diabetes comprising the step of detecting the presence, absence, or quantity of a translation product of a transcript having a sequence selected from the group consisting of: SEQ ID NO: 2-4;6-9;11;13-18;20-21 in a sample. The presence or quantity of said translation product is indicative of obesity and/or diabetes

The present invention relates to kits for screening, diagnosing and monitoring an individual for obesity and/or diabetes.

The present invention relates to proteins encoded by a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 2-4;6-9;11;13-18;20-21 and immunogenic fragments thereof.

The present invention relates to antibodies which specifically bind to an epitope on a protein encoded by a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 2-4;6-9;11;13-18;20-21.

The present invention relates to antibodies which specifically bind to an epitope on a protein encoded by a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 2-4;6-9;11;13-18;20-21 that are linked to detectable labels or active agents.

The present invention relates to pharmaceutical composition comprising antibodies which specifically bind to an epitope on a protein encoded by a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 2-4;6-9;11;13-18;20-21 that are linked to active agents.

The present invention relates to methods of treating an individual suspected of suffering from obesity and/or diabetes. The methods comprise the step of administering to individuals antibodies which specifically bind to an epitope on a protein encoded by a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 2-4;6-9;11;13-18;20-21 that are linked to active agents.

The present invention relates to methods of delivering a nucleic acid molecule to obesity and/or diabetic cell of an individual. The methods comprise the step of administering to said individual a pharmaceutical composition comprising antibodies which specifically bind to an epitope on a protein encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2-4;6-9;11;13-18;20-21 and,
a nucleic acid molecules.

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 shows multiple alignment of four amino acid sequences ID NOS: 22-25 of human origin (depicted in SEQ ID NO:22 to SEQ ID NO:25 to each other and to the original sequence;

Fig. 2 shows multiple alignment of five amino acid sequences ID NOS: 26-30 of mouse origin (depicted in SEQ ID NO:26 to SEQ ID NO:30 to each other and to the original sequence;

Fig. 3 shows alignment of two amino acid sequences ID NOS: 31-32 of human origin (depicted in SEQ ID NO:31 to SEQ ID NO:32 to the original sequence;

Fig. 4 shows multiple alignment of seven amino acid sequences ID NOS: 33-39 of human origin (depicted in SEQ ID NO:33 to SEQ ID NO:39 to each other and to the original sequence;

Fig. 5 shows multiple alignment of three amino acid sequences ID NOS: 40-42 of human origin (depicted in SEQ ID NO:40 to SEQ ID NO:42 to each other and to the original sequence;

Fig. 6 shows multiple alignment of four nucleic acid sequences ID NOS: 1-4 of human origin (depicted in SEQ ID NO:1 to SEQ ID NO:4 to each other and to the original sequence;

Fig. 7 shows multiple alignment of five nucleic acid sequences ID NOS: 5-9 of mouse origin (depicted in SEQ ID NO:5 to SEQ ID NO:9 to each other and to the original sequence;

Fig. 8 shows alignment of two nucleic acid sequences ID NOS: 10-11 of human origin (depicted in SEQ ID NO:10 to SEQ ID NO:11 to the original sequence;

Fig. 9 shows multiple alignment of seven nucleic acid sequences ID NOS: 12-18 of human origin (depicted in SEQ ID NO:12 to SEQ ID NO:18 to each other and to the original sequence;

Fig. 10 shows multiple alignment of three amino acid sequences ID NOS: 19-21 of human origin (depicted in SEQ ID NO:19 to SEQ ID NO:21 to each other and to the original sequence;

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example I: Obesity and/or diabetes variants nucleic acid sequence

The nucleic acid sequences of the invention include nucleic acid sequences which encode Obesity and/or diabetes variants products and fragments and analogs thereof. The nucleic acid sequences may alternatively be sequences complementary to the above coding sequences, or to regions of said coding sequence. The length of the complementary sequences is sufficient to avoid the expression of the coding sequence. The nucleic acid sequences may be in the

form of RNA or in the form of DNA, and include messenger RNA, synthetic RNA and DNA, cDNA, and genomic DNA. The DNA may be double-stranded or single-stranded, and if single-stranded may be the coding strand or the non-coding (anti-sense, complementary) strand. The nucleic acid sequences may also both include dNTPs, rNTPs as well as non naturally occurring sequences. The sequence may also be a part of a hybrid between an amino acid sequence and a nucleic acid sequence.

In a general embodiment, the nucleic acid sequence has at least 90%, identity with any one of the sequence identified as SEQ ID NO:2 to SEQ ID NO:4 or SEQ ID NO:6 to SEQ ID NO:9 or SEQ ID NO:11 or SEQ ID NO:13 to SEQ ID:18 or SEQ ID NO:20 to SEQ ID 21.

The nucleic acid sequences may include the coding sequence by itself. By another alternative the coding region may be in combination with additional coding sequences, such as those coding for fusion protein or signal peptides, in combination with non-coding sequences, such as introns and control elements, promoter and terminator elements or 5' and/or 3' untranslated regions, effective for expression of the coding sequence in a suitable host, and/or in a vector or host environment in which the variant nucleic acid sequences is introduced as a heterologous sequence.

The nucleic acid sequences of the present invention may also have the Obesity and/or diabetes variants products coding sequences fused in-frame to a marker sequence which allows for purification of the variant product. The marker sequence may be, for example, a hexahistidine tag to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., *et al. Cell* **37**:767 (1984)).

Also included in the scope of the invention are fragments as defined above also referred to herein as oligonucleotides, typically having at least 20 bases,

preferably 20-30 bases corresponding to a region of the coding-sequence nucleic acid sequence. The fragments may be used as probes, primers, and when complementary also as antisense agents, and the like, according to known methods.

As indicated above, the nucleic acid sequence may be substantially a depicted in SEQ ID NO:2 to SEQ ID NO:4 or SEQ ID NO:6 to SEQ ID NO:9 or SEQ ID NO:11 or SEQ ID NO:13 to SEQ ID:18 or SEQ ID NO:20 to SEQ ID 21 or fragments thereof or sequences having at least 90% identity to the above sequence as explained above. Alternatively, due to the degenerative nature of the genetic code, the sequence may be a sequence coding for any one of the amino acid sequence of SEQ ID NO:23 to SEQ ID NO:25 or SEQ ID NO:27 to SEQ ID NO:30 or SEQ ID NO:32 or SEQ ID NO:34 to SEQ ID:39 or SEQ ID NO:41 to SEQ ID 42, or fragments or analogs of said amino acid sequence.

A. Preparation of nucleic acid sequences

The nucleic acid sequences may be obtained by screening cDNA libraries using oligonucleotide probes which can hybridize to or PCR-amplify nucleic acid sequences which encode the Obesity and/or diabetes variants products disclosed above. cDNA libraries prepared from a variety of tissues are commercially available and procedures for screening and isolating cDNA clones are well-known to those of skill in the art. Such techniques are described in, for example, Sambrook *et al.* (1989) Molecular Cloning: A Laboratory Manual (2nd Edition), Cold Spring Harbor Press, Plainview, N.Y. and Ausubel FM et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y.

The nucleic acid sequences may be extended to obtain upstream and downstream sequences such as promoters, regulatory elements, and 5' and 3' untranslated regions (UTRs). Extension of the available transcript sequence may be performed by numerous methods known to those of skill in the art, such as PCR or primer extension (Sambrook *et al., supra*), or by the RACE method using, for example, the Marathon RACE kit (Clontech, Cat. # K1802-1).

Alternatively, the technique of *"restriction-site"* PCR (Gobinda *et al. PCR Methods Applic.* **2**:318-22, (1993)), which uses universal primers to retrieve flanking sequence adjacent a known locus, may be employed. First, genomic DNA is amplified in the presence of primer to a linker sequence and a primer specific to the known region. The amplified sequences are subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR can be used to amplify or extend sequences using divergent primers based on a known region (Triglia, T. *et al.*, *Nucleic Acids Res.* **16**:8186, (1988)). The primers may be designed using OLIGO(R) 4.06 Primer Analysis Software (1992; National Biosciences Inc, Plymouth, Minn.), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68-72°C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Capture PCR (Lagerstrom, M. *et al., PCR Methods Applic.* **1**:111-19, (1991)) is a method for PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA. Capture PCR also requires multiple restriction enzyme digestions and ligations to place an engineered double-stranded sequence into a flanking part of the DNA molecule before PCR.

Another method which may be used to retrieve flanking sequences is that of Parker, J.D., *et al., Nucleic Acids Res.*, **19**:3055-60, (1991)). Additionally, one can use PCR, nested primers and PromoterFinder™ libraries to *"walk in"* genomic DNA (PromoterFinder™; Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions. Preferred libraries for screening for full length cDNAs are ones that have been size-selected

to include larger cDNAs. Also, random primed libraries are preferred in that they will contain more sequences which contain the 5' and upstream regions of genes.

A randomly primed library may be particularly useful if an oligo d(T) library does not yield a full-length cDNA. Genomic libraries are useful for extension into the 5' nontranslated regulatory region.

The nucleic acid sequences and oligonucleotides of the invention can also be prepared by solid-phase methods, according to known synthetic methods. Typically, fragments of up to about 100 bases are individually synthesized, then joined to form continuous sequences up to several hundred bases.

B. Use of Obesity and/or diabetes variants nucleic acid sequences for the production of Obesity and/or diabetes variants products

In accordance with the present invention, nucleic acid sequences specified above may be used as recombinant DNA molecules that direct the expression of Obesity and/or diabetes variant products.

As will be understood by those of skill in the art, it may be advantageous to produce Obesity and/or diabetes variants product-encoding nucleotide sequences possessing codons other than those which appear in SEQ ID NO:2 to SEQ ID NO:4 or SEQ ID NO:6 to SEQ ID NO:9 or SEQ ID NO:11 or SEQ ID NO:13 to SEQ ID:18 or SEQ ID NO:20 to SEQ ID 21 which are those which naturally occur in the human genome. Codons preferred by a particular prokaryotic or eukaryotic host (Murray, E. *et al. Nuc Acids Res.*, **17**:477-508, (1989)) can be selected, for example, to increase the rate of variant product expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

The nucleic acid sequences of the present invention can be engineered in order to alter a Obesity and/or diabetes variants products coding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the product. For example, alterations

may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, etc.

The present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which nucleic acid sequences of the invention have been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the constructs further comprise regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are also described in Sambrook, *et al., (supra)*.

The present invention also relates to host cells which are genetically engineered with vectors of the invention, and the production of the product of the invention by recombinant techniques. Host cells are genetically engineered (i.e., transduced, transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the expression of the variant nucleic acid sequence. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art.

The nucleic acid sequences of the present invention may be included in any one of a variety of expression vectors for expressing a product. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phagé DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However,

any other vector may be used as long as it is replicable and viable in the host. The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and related sub-cloning procedures are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate transcription control sequence (promoter) to direct mRNA synthesis. Examples of such promoters include: LTR or SV40 promoter, the *E.coli lac* or *trp* promoter, the phage lambda *PL* promoter, and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vectors also contains a ribosome binding site for translation initiation, and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E.coli.*

The vectors containing the appropriate DNA sequence as described above, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein. Examples of appropriate expression hosts include: bacterial cells, such as *E.coli*, *Streptomyces, Salmonella typhimurium*; fungal cells, such as yeast; insect cells such as *Drosophila* and *Spodoptera* Sf9; animal cells such as CHO, COS, HEK 293 or Bowes melanoma; adenoviruses; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein. The invention is not limited by the host cells employed.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the Obesity and/or diabetes variant product. For example, when large quantities of Obesity and/or diabetes variant product

are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be desirable. Such vectors include, but are not limited to, multifunctional *E.coli* cloning and expression vectors such as *Bluescript*(R) (Stratagene), in which the Obesity and/or diabetes variants polypeptides coding sequence may be ligated into the vector in-frame with sequences for the amino-terminal Met and the subsequent 7 residues of beta-galactosidase so that a hybrid protein is produced; *pIN* vectors (Van Heeke & Schuster *J. Biol. Chem.* **264**:5503-5509, (1989)); *pET* vectors (Novagen, Madison WI); and the like.

In the yeast *Saccharomyces cerevisiae* a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH may be used. For reviews, see Ausubel *et al. (supra)* and Grant *et al.*, (*Methods in Enzymology* **153**:516-544, (1987)).

In cases where plant expression vectors are used, the expression of a sequence encoding variant products may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of *CaMV* (Brisson *et al., Nature* **310**:511-514. (1984)) may be used alone or in combination with the omega leader sequence from TMV (Takamatsu *et al.*, *EMBO J.*, **6**:307-311, (1987)). Alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi *et al.*, *EMBO J.* **3**:1671-1680, (1984); Broglie *et al.*, *Science* **224**:838-843, (1984)); or heat shock promoters (Winter J and Sinibaldi R.M., *Results Probl. Cell Differ.*, **17**:85-105, (1991)) may be used. These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. For reviews of such techniques, see Hobbs S. or Murry L.E. (1992) in McGraw Hill Yearbook of Science and Technology, McGraw Hill, New York, N.Y., pp 191-196; or Weissbach and Weissbach (1988) *Methods for Plant Molecular Biology*, Academic Press, New York, N.Y., pp 421-463.

Obesity and/or diabetes variants products may also be expressed in an insect system. In one such system, Autographa californica nuclear polyhedrosis

virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The Obesity and/or diabetes variants products coding sequence may be cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of Obesity and/or diabetes coding sequences will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which variant protein is expressed (Smith *et al., J. Virol.* 46:584, (1983); Engelhard, E.K. *et al., Proc. Nat. Acad. Sci.* **91**:3224-7, (1994)).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, Obesity and/or diabetes variants products coding sequences may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a nonessential E1 or E3 region of the viral genome will result in a viable virus capable of expressing variant protein in infected host cells (Logan and Shenk, *Proc. Natl. Acad. Sci.* **81**:3655-59, (1984). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be required for efficient translation of variants products coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where Obesity and/or diabetes variants products coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use

(Scharf, D. *et al.,* (1994) *Results Probl. Cell Differ.,* **20**:125-62, (1994); Bittner et al., *Methods in Enzymol* **153**:516-544, (1987)).

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., and Battey, I. (1986) Basic Methods in Molecular Biology). Cell-free translation systems can also be employed to produce polypeptides using RNAs derived from the DNA constructs of the present invention.

A host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the protein include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a *"pre-pro"* form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, WI38, etc. have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express variant products may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the

introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler M., *et al.*, *Cell* **11**:223-32, (1977)) and adenine phosphoribosyltransferase (Lowy I., *et al.*, *Cell* **22**:817-23, (1980)) genes which can be employed in *tk-* or *aprt-* cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, *dhfr* which confers resistance to methotrexate (Wigler M., *et al., Proc. Natl. Acad. Sci.* **77**:3567-70, (1980)); *npt*, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. *et al., J. Mol. Biol.*, **150**:1-14, (1981)) and *als* or *pat*, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, *trpB*, which allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman S.C. and R.C. Mulligan, *Proc. Natl. Acad. Sci.* **85**:8047-51, (1988)). The use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate, GUS, and luciferase and its substrates, luciferin and ATP, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C.A. *et. al., Methods Mol. Biol.,* 55:121-131, (1995)).

Host cells transformed with nucleotide sequences encoding Obesity and/or diabetes variants products may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The product produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing nucleic acid sequences encoding Obesity and/or diabetes variants products can be designed with signal

sequences which direct secretion of Obesity and/or diabetes variants products through a prokaryotic or eukaryotic cell membrane.

The Obesity and/or diabetes variants products may also be expressed as recombinant proteins with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, Wash.). The inclusion of a protease-cleavable polypeptide linker sequence between the purification domain and Obesity and/or diabetes variants products is useful to facilitate purification. One such expression vector provides for expression of a fusion protein compromising a variant polypeptide fused to a polyhistidine region separated by an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography, as described in Porath, *et al.*, *Protein Expression and Purification,* **3**:263-281, (1992)) while the enterokinase cleavage site provides a means for isolating variant polypeptide from the fusion protein. *pGEX* vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to ligand-agarose beads (e.g., glutathione-agarose in the case of GST-fusions) followed by elution in the presence of free ligand.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling,

sonication, mechanical disruption, or use of cell lysing agents, or other methods, which are well know to those skilled in the art.

The Obesity and/or diabetes variants products can be recovered and purified from recombinant cell cultures by any of a number of methods well known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

3rd. Use of varient to produce proteins

C.1 Separation:

Sf-9 cells were infected with Obesity and/or diabetes variants expressing baculovirus (Ac-obesity and/or diabetes variant) comprising the amino acid sequence of SEQ ID NO:23 to SEQ ID NO:25 or SEQ ID NO:27 to SEQ ID NO:30 or SEQ ID NO:32 or SEQ ID NO:34 to SEQ ID:39 or SEQ ID NO:41 to SEQ ID 42 at MOI of 2. The cells were grown in 28°C at continuous shaking (90rpm). At 60 hours post infection (hpi) the medium was collected and cells were separated from the medium by centrifugation at 5000RPM for 5 minutes. 10ml medium was separated using cation exchange chromatography with SP-Sepharose column. The Column was equilibrated with PBS pH-6.5 and following loading of the sample on the column the column was washed with PBS to elute the unbound proteins (flow through fraction). Elution was done with increasing concentration of NaCl at flow rate of 2ml/min (5%NaCl/min).

The different fractions were subjected to SDS-PAGE electrophoresis and to western blotting using anti m Obesity and/or diabetes variant antibody.

C.1 Secretion:

Sf-9 cells were infected with Obesity and/or diabetes variants expressing baculovirus (Ac-obesity and/or diabetes variant) at MOI of 2. The cells were grown at 28°C at continuous shaking (90rpm) and 1ml samples were collected at 24, 48 and 60 hours post infection (hpi). Following centrifugation Cells pellet was lysed with lysis buffer (50mM Tris pH 7.5, 1% triton X100, and protease inhibitor cocktail) at 4°C for 30 min and sonicated for 30 seconds. The sample was centrifuged for 10 minutes at 14000rmp and the sup was designated Pellet. 40 µl of the pellet preparation and of the medium (Designated Medium) were supplemented with sample buffer and electrophoreses on a 15% SDS-PAGE. Following electrophoresis the gel was subjected to a semi dry protein transfer onto a nitrocellulose membrane. The membrane was incubated with anti m Obesity and/or diabetes variants antibody for 2 hours and with secondary anti rabbit antibody for an additional 1 hour.

Detection of the signal was done using a commercial western blot detection kit.

D. Diagnostic applications utilizing nucleic acid sequences

The nucleic acid sequences of the present invention may be used for a variety of diagnostic purposes. The nucleic acid sequences may be used to detect and quantitate expression of the Obesity and/or diabetes variant in patient's cells, e.g. biopsied tissues, by detecting the presence of mRNA coding for the Obesity and/or diabetes variants products. Alternatively, the assay may be used to detect the soluble variants in the serum or blood. This assay typically involves obtaining total mRNA from the tissue or serum and contacting the mRNA with a nucleic acid probe. The probe is a nucleic acid molecule of at least 20 nucleotides, preferably 20-30 nucleotides, capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding the Obesity and/or diabetes variant product under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of variant. This assay can be used to distinguish between absence, presence, and

excess expression of Obesity and/or diabetes variants products and to monitor levels of Obesity and/or diabetes variants expression during therapeutic intervention. In addition, the assay may be used to compare the levels of the Obesity and/or diabetes variant of the invention to the levels of the original Obesity and/or diabetes sequence from which it has been varied or to levels of each other, which comparison may have some physiological meaning.

The invention also contemplates the use of the nucleic acid sequences as a diagnostic for diseases resulting from inherited defective variants sequences, or diseases in which the ratio of the amount of the original Obesity and/or diabetes sequence from which the Obesity and/or diabetes variants were varied to the novel Obesity and/or diabetes variants of the invention is altered. These sequences can be detected by comparing the sequences of the defective (i.e., mutant) Obesity and/or diabetes variants coding region with that of a normal coding region. Association of the sequence coding for mutant Obesity and/or diabetes variants products with abnormal variants products activity may be verified. In addition, sequences encoding mutant Obesity and/or diabetes variants products can be inserted into a suitable vector for expression in a functional assay system (e.g., colorimetric assay, complementation experiments in a variant protein deficient strain of HEK293 cells) as yet another means to verify or identify mutations. Once mutant genes have been identified, one can then screen populations of interest for carriers of the mutant gene.

Individuals carrying mutations in the nucleic acid sequences of the present invention may be detected at the DNA level by a variety of techniques. Nucleic acids used for diagnosis may be obtained from a patient's cells, including but not limited to such as from blood, urine, saliva, placenta, tissue biopsy and autopsy material. Genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki, *et al.*, *Nature* **324**:163-166, (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid of the present invention can be used to identify and analyze mutations in the gene of the present invention.

Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype.

Point mutations can be identified by hybridizing amplified DNA to radiolabeled RNA of the invention or alternatively, radiolabeled antisense DNA sequences of the invention. Sequence changes at specific locations may also be revealed by nuclease protection assays, such RNase and S1 protection or the chemical cleavage method (e.g. Cotton, *et alProc. Natl. Acad. Sci.* USA, **85**:4397-4401, (1985)), or by differences in melting temperatures. *"Molecular beacons"* (Kostrikis L.G. *et al.*, Science **279**:1228-1229, (1998)), hairpin-shaped, single-stranded synthetic oligo- nucleotides containing probe sequences which are complementary to the nucleic acid of the present invention, may also be used to detect point mutations or other sequence changes as well as monitor expression levels of variant product. Such diagnostics would be particularly useful for prenatal testing.

Another method for detecting mutations uses two DNA probes which are designed to hybridize to adjacent regions of a target, with abutting bases, where the region of known or suspected mutation(s) is at or near the abutting bases. The two probes may be joined at the abutting bases, e.g., in the presence of a ligase enzyme, but only if both probes are correctly base paired in the region of probe junction. The presence or absence of mutations is then detectable by the presence or absence of ligated probe.

Also suitable for detecting mutations in the Obesity and/or diabetes variants products coding sequences are oligonucleotide array methods based on sequencing by hybridization (SBH), as described, for example, in U.S. Patent No. 5,547,839. In a typical method, the DNA target analyte is hybridized with an array of oligonucleotides formed on a microchip. The sequence of the target can then be *"read"* from the pattern of target binding to the array.

E. Therapeutic applications of nucleic acid sequences

Nucleic acid sequences of the invention may also be used for therapeutic purposes. Turning first to the second aspect of the invention (i.e. inhibition of expression of Obesity and/or diabetes variants), expression of Obesity and/or diabetes variants products may be modulated through antisense technology, which controls gene expression through hybridization of complementary nucleic acid sequences, i.e. antisense DNA or RNA, to the control, 5' or regulatory regions of the gene encoding variant product. For example, the 5' coding portion of the nucleic acid sequence sequence which codes for the product of the present invention is used to design an antisense oligonucleotide of from about 10 to 40 base pairs in length. Oligonucleotides derived from the transcription start site, e.g. between positions -10 and +10 from the start site, are preferred. An antisense DNA oligonucleotide is designed to be complementary to a region of the nucleic acid sequence involved in transcription (Lee *et al., Nucl. Acids, Res.*, **6**:3073, (1979); Cooney et al., Science **241**:456, (1988); and Dervan *et al., Science* **251**:1360, (1991)), thereby preventing transcription and the production of the variant products. An antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into the variant products (Okano *J. Neurochem.* **56**:560, (1991)). The antisense constructs can be delivered to cells by procedures known in the art such that the antisense RNA or DNA may be expressed *in vivo*. The antisense may be antisense mRNA or DNA sequence capable of coding such antisense mRNA. The antisense mRNA or the DNA coding thereof can be complementary to the full sequence of nucleic acid sequences coding for the Obesity and/or diabetes variant protein or to a fragment of such a sequence which is sufficient to inhibit production of a protein product. Antisense technologies can also be used for inhibiting expression of one variant as compared to the other, or inhibiting the expression of the variant/s as compared to the original sequence.

Turning now to the first aspect of the invention, i.e. expression of Obesity and/or diabetes variants, expression of Obesity and/or diabetes variants products may be increased by providing coding sequences for coding for said Obesity

and/or diabetes variants products under the control of suitable control elements ending its expression in the desired host.

The nucleic acid sequences of the invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The products of the invention may also be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as *"gene therapy."* Cells from a patient may be engineered with a nucleic acid sequence (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered in vivo for expression of a polypeptide *in vivo* by procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptides of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering products of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells *in vivo* after combination with a suitable delivery vehicle.

Retroviruses from which the retroviral plasmid vectors mentioned above may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma

Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the *PE501*, *PA317*, *psi-2*, *psi-AM*, *PA12*, *T19-14X*, *VT-19-17-H2*, *psi-CRE*, *psi-CRIP*, *GP+E-86*, *GP+envAm12*, and *DAN* cell lines as described in Miller (*Human Gene Therapy*, Vol. **1**, pg. 5-14, (1990)). The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

The genes introduced into cells may be placed under the control of inducible promoters, such as the radiation-inducible Egr-1 promoter, (Maceri, H.J., *et al., Cancer Res.*, **56**(19):4311 (1996)), to stimulate variant production or antisense inhibition in response to radiation, eg., radiation therapy for treating tumors.

Example II. Obesity and/or diabetes Variants products

The substantially purified Obesity and/or diabetes variant product of the invention has been defined above as the product coded from the nucleic acid sequence of the invention. Preferably the amino acid sequence is an amino acid

sequence having at least 90% identity the sequence identified as SEQ ID NO:23 to SEQ ID NO:25 or SEQ ID NO:27 to SEQ ID NO:30 or SEQ ID NO:32 or SEQ ID NO:34 to SEQ ID:39 or SEQ ID NO:41 to SEQ ID 42. The protein or polypeptide may be in mature and/or modified form, also as defined above, for example, modified by cleavage of the leader sequence. Also contemplated are protein fragments having at least 10 contiguous amino acid residues, preferably at least 10-20 residues, derived from the Obesity and/or diabetes variant products, as well as homologues as explained above.

The sequence variations are preferably those that are considered conserved substitutions, as defined above. Thus, for example, a protein with a sequence having at least 90% sequence identity with the products identified as SEQ ID NO:23 to SEQ ID NO:25 or SEQ ID NO:27 to SEQ ID NO:30 or SEQ ID NO:32 or SEQ ID NO:34 to SEQ ID:39 or SEQ ID NO:41 to SEQ ID 42, preferably by utilizing conserved substitutions as defined above is also part of the invention, and provided that it is not identical to the original peptide from which it has been varied (typically the substitutions are in regions where the variant differs from the original sequence as for example in Table 1). In a more specific embodiment, the protein has or contains the sequence identified SEQ ID NO:23 to SEQ ID NO:25 or SEQ ID NO:27 to SEQ ID NO:30 or SEQ ID NO:32 or SEQ ID NO:34 to SEQ ID:39 or SEQ ID NO:41 to SEQ ID 42. The Obesity and/or diabetes variants products may be (i) one in which one or more of the amino acid residues in a sequence listed above are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue), or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the Obesity and/or diabetes variants products is fused with another compound, such as a compound to increase the half-life of the protein (for example, polyethylene glycol (PEG)), or a moiety which serves as targeting means to direct the protein to its target tissue or target cell population (such as an antibody), or (iv) one in which additional amino acids are fused to the Obesity and/or diabetes variant product. Such fragments, variants and derivatives are

deemed to be within the scope of those skilled in the art from the teachings herein.

A. Preparation of Obesity and/or diabetes variants products

Recombinant methods for producing and isolating the Obesity and/or diabetes variant products, and fragments of the protein are described above.

In addition to recombinant production, fragments and portions of variant products may be produced by direct peptide synthesis using solid-phase techniques (cf. Stewart *et al.*, (1969) Solid-Phase Peptide Synthesis, WH Freeman Co, San Francisco; Merrifield J., *J. Am. Chem. Soc.*, **85**:2149-2154, (1963)). In vitro peptide synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City, Calif.) in accordance with the instructions provided by the manufacturer. Fragments of Obesity and/or diabetes variants products may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

2nd. Therapeutic uses and compositions utilizing the Obesity and/or diabetes variants products

The Obesity and/or diabetes variants products of the invention are generally useful in treating obesity and/or diabetes.

Obesity and/or diabetes variant products or fragments may be administered by any of a number of routes and methods designed to provide a consistent and predictable concentration of compound at the target organ or tissue. The product-containing compositions may be administered alone or in combination with other agents, such as stabilizing compounds, and/or in combination with other pharmaceutical agents such as drugs or hormones.

Obesity and/or diabetes variants product-containing compositions may be administered by a number of routes including, but not limited to oral,

intravenous, intramuscular, transdermal, subcutaneous, topical, sublingual, or rectal means as well as by nasal application. Obesity and/or diabetes variant product-containing compositions may also be administered via liposomes. Such administration routes and appropriate formulations are generally known to those of skill in the art.

The Obesity and/or diabetes variants products can be given via intravenous or intraperitoneal injection. Similarly, the product may be injected to other localized regions of the body. The product may also be administered via nasal insufflation. Enteral administration is also possible. For such administration, the product should be formulated into an appropriate capsule or elixir for oral administration, or into a suppository for rectal administration.

The foregoing exemplary administration modes will likely require that the product be formulated into an appropriate carrier, including ointments, gels, suppositories. Appropriate formulations are well known to persons skilled in the art.

Dosage of the product will vary, depending upon the potency and therapeutic index of the particular polypeptide selected.

A therapeutic composition for use in the treatment method can include the product in a sterile injectable solution, the polypeptide in an oral delivery vehicle, the product in an aerosol suitable for nasal administration, or the product in a nebulized form, all prepared according to well known methods. Such compositions comprise a therapeutically effective amount of the compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The product of the invention may also be used to modulate endothelial differentiation and proliferation as well as to modulate apoptosis either *ex vivo* or *in vitro*, for example, in cell cultures.

Example III. Anti-variant antibodies

A. Synthesis

In still another aspect of the invention, the purified variants products are used to produce anti-variant antibodies which have diagnostic and therapeutic uses related to the activity, distribution, and expression of the Obesity and/or diabetes variants products.

Antibodies to the Obesity and/or diabetes variant may be generated by methods well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, humanized, single chain, Fab fragments and fragments produced by an Fab expression library. Antibodies, i.e., those which inhibit dimer formation, are especially preferred for therapeutic use.

A fragment of the Obesity and/or diabetes variants products for antibody induction is not required to feature biological activity but has to feature immunological activity; however, the protein fragment or oligopeptide must be antigenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of at least five amino acids, preferably at least 10 amino acids of the sequences specified in SEQ ID NO:23 to SEQ ID NO:25 or SEQ ID NO:27 to SEQ ID NO:30 or SEQ ID NO:32 or SEQ ID NO:34 to SEQ ID:39 or SEQ ID NO:41 to SEQ ID 42. Preferably they should mimic a portion of the amino acid sequence of the natural protein and may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of Obesity and/or diabetes variants proteins amino acids may be fused with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule. Procedures well known in the art can be used for the production of antibodies to Obesity and/or diabetes variants products.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, etc may be immunized by injection with Obesity and/or diabetes variants products or any portion, fragment or oligopeptide which retains immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include but are not limited to Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil

emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are potentially useful human adjuvants.

Monoclonal antibodies to Obesity and/or diabetes variants protein may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Koehler and Milstein (*Nature* **256**:495-497, (1975)), the human B-cell hybridoma technique (Kosbor *et al., Immunol. Today* **4**:72, (1983); Cote *et al., Proc. Natl. Acad. Sci.* **80**:2026-2030, (1983)) and the EBV-hybridoma technique (Cole, *et al., Mol. Cell Biol.* 62:109-120, (1984)).

Techniques developed for the production of *"chimeric antibodies"*, the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can also be used (Morrison *et al., Proc. Natl. Acad. Sci.* **81**:6851-6855, (1984); Neuberger *et al., Nature* **312**:604-608, (1984); Takeda *et al., Nature* **314**:452-454, (1985)). Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce single-chain antibodies specific for the variant protein.

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi *et al.* (*Proc. Natl. Acad. Sci.* **86**:3833-3837, 1989)), and Winter G and Milstein C., (*Nature* **349**:293-299, (1991)).

Antibody fragments which contain specific binding sites for the Obesity and/or diabetes variant protein may also be generated. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy

identification of monoclonal Fab fragments with the desired specificity (Huse W.D. *et al., Science* 256:1275-1281, (1989)).

B. Diagnostic applications of antibodies

A variety of protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the formation of complexes between the Obesity and/or diabetes variants products and its specific antibody and the measurement of complex formation. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two noninterfering epitopes on a specific variant product is preferred, but a competitive binding assay may also be employed. These assays are described in Maddox D.E., *et al.,* (*J. Exp. Med.* **158**:1211, (1983)).

Antibodies which specifically bind the Obesity and/or diabetes variant product are useful for the diagnosis of conditions or diseases characterized by expression of the novel Obesity and/or diabetes variants of the invention (where normally it is not expressed) by over or under expression of Obesity and/or diabetes variants as well as for detection of diseases in which the proportion between the amount of the Obesity and/or diabetes variants of the invention and the original Obesity and/or diabetes sequence from which it varied is altered. Alternatively, such antibodies may be used in assays to monitor patients being treated with Obesity and/or diabetes variants products. Diagnostic assays for variants proteins include methods utilizing the antibody and a label to detect variants products in human body fluids or extracts of cells or tissues. The products and antibodies of the present invention may be used with or without modification. Frequently, the proteins and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of reporter molecules are known in the art.

A variety of protocols for measuring the Obesity and/or diabetes variants products, using either polyclonal or monoclonal antibodies specific for the

respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescent activated cell sorting (FACS). As noted above, a two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on Obesity and/or diabetes variants products is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, *et al. (supra).* Such protocols provide a basis for diagnosing altered or abnormal levels of Obesity and/or diabetes variants products expression. Normal or standard values for Obesity and/or diabetes variants products expression are established by combining body fluids or cell extracts taken from normal subjects, preferably human, with antibodies to Obesity and/or diabetes variants products under conditions suitable for complex formation which are well known in the art. The amount of standard complex formation may be quantified by various methods, preferably by photometric methods. Then, standard values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by disease. Deviation between standard and subject values establishes the presence of disease state.

The antibody assays are useful to determine the level of Obesity and/or diabetes variants products present in a body fluid sample, in order to determine whether it is being expressed at all, whether it is being overexpressed or underexpressed in the tissue, or as an indication of how Obesity and/or diabetes variants levels of variable products are responding to drug treatment.

3rd. Therapeutic uses of antibodies

In addition to their diagnostic use the antibodies may have a therapeutical utility in blocking or decreasing the activity of the obesity and/or diabetes variants products in pathological conditions where beneficial effect can be achieved by such a decrease.

The antibody employed is preferably a humanized monoclonal antibody, or a human Mab produced by known globulin-gene library methods. The antibody is administered typically as a sterile solution by IV injection, although other parenteral routes may be suitable. Typically, the antibody is administered in an amount between about 1-15 mg/kg body weight of the subject. Treatment is continued, e.g., with dosing every 1-7 days, until a therapeutic improvement is seen.

Although the invention has been described with reference to specific methods and embodiments, it is appreciated that various modifications and changes may be made without departing from the invention.